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STUDIES OF THE EPIDEMIOLOGY AND CONTROL OF FIREBLIGHT OF APPLE[1]

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Fireblight, caused by *Bacillus amylovorus* (Burr.) Trev., is one of the most destructive diseases of pomaceous fruits. It has been recognized in America for more than 125 years and has been the subject of much investigation for a half century. During the period from 1877 to 1882 Burrill (5, 6, 7) discovered the bacterial nature of the disease and named the causal organism. Arthur (3) isolated the organism and by inoculation tests further established its causal relationship to the disease. He also recommended as a control measure the cutting out of blighted parts of the host plant. Waite (40, 41, 42) showed that the blight organism is spread from blossom to blossom by bees and that the disease might be controlled by means of an adequate program of excision of diseased parts where the work covered a sufficiently large area. From time to time experiments and observations have been made upon the modes of dissemination of blight. Stewart (36, 37, 38), Burrill (4), Jones (24, 25), and others demonstrated the spread of twig blight by means of sucking and boring insects. Stevens (34) suggested the possibility of the spread of the disease through windborne inoculum, and Gossard and Walton (17) showed that rain is a factor, at least locally, in the dissemination of blight. During the past 15 years much work has been done in an attempt to improve methods of blight control. Reimer (31) and Day (10, 11) have contributed toward the betterment of methods of excising blighted parts. Reimer (30) has made sub-

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stantial progress in combating pear blight by the use of disease resistant stock.

In Wisconsin, epidemic outbreaks of fireblight of apple are common and destructive. Numerous attempts on the part of apple growers of this State to control the disease by the excision method have met with indifferent success. Whether this is to be attributed to improper execution or to inadequacies of the method under Wisconsin conditions has been subject to question. In view of these facts further investigations under local conditions appeared to be desirable. This work was directed along two major lines: (a) a detailed study of the seasonal development of the fireblight organism and of the disease in relation to host development and to certain environmental factors, and (b) tests of certain control measures. The work was carried on at Madison, Wisconsin, during the winters 1923–24 and 1924–25 and in field laboratories located in orchards near Menomonie and Gays Mills during the growing seasons of 1924 and 1925.

STUDIES OF THE OVERWINTERING OF BACILLUS AMYLOVORUS

A more detailed knowledge of the mode and abundance of overwintering of the blight organism under Wisconsin conditions seemed fundamental to an adequate understanding of the epidemiology and control of the disease. Consequently the following studies were initiated.

Overwintering on wild hosts

A study of wild hosts of *B. amylovorus* in the vicinity of the experimental orchards, chiefly *Pyrus coronaria* L. and *Crataegus* spp., did not reveal any cases of overwintering of the bacteria. In view of this fact and of the abundance of overwintering in the commercial orchards, as reported below, wild hosts are not considered to have played any significant part in overwintering the organism under the conditions of these studies. It should be noted, however, that wild hosts of *B. amylovorus* are comparatively sparse in their occurrence in Wisconsin.

Variety of apple in relation to overwintering

One of the first problems of overwintering was to learn the relative importance of various commercial varieties in carrying over the organism from season to season. With this in mind, observations were made in the early spring of 1924 at Weston Orchards and at the orchard of the State Horticultural Society near Menomonie, and in 1925 at Kickapoo Orchards near Gays Mills, Wisconsin.

The chief criterion of overwintering was the production of exudate on cankers and twigs which had been blighted the previous year, although the

extension of blighted areas was also used to a less extent. In the latter case, where there was no production of exudate, the identification of the active cankers was confirmed by isolating the fireblight organism from the extending margins. Of 24 varieties of apple observed, the following were found to overwinter the organism and are listed in the order of their importance in this respect in the situations studied: Transcendent, Hyslop, Yellow Transparent, McMahon, Wealthy, Fameuse, Tolman Sweet, McIntosh, and Dudley.

Overwintering in commercial apple orchards

Studies of overwintering of the blight organism in commercial orchards were initiated with the aim of gaining a clearer understanding of the types of lesion associated with carrying the bacteria through the winter, the host tissues in which overwintering ordinarily occurs, and the quantitative aspect of overwintering in relation to lesions on cankers, twigs and fruits.

Studies of cankers: Location of organism. On the basis of work done on pear, Jones (24) states that the blight organisms . . . "live over winter, not in the dead cankered tissue, but in the living bark immediately surrounding the cankered area." By studying cankers on apple during the late fall, winter, and early spring the present writer sought to gain a clearer conception of the relation of the overwintering organism to the tissues of this host.

During the fall and winter of the years 1923–24 and 1924–25 fireblight material was sent to Madison from the experimental orchards for isolation studies. This material consisted of blighted twigs ¼ to ½ inch in diameter, cankered branches ¾ to 2 inches in diameter with 8 to 12 inches of apparently healthy bark above and below the cankers, and mummified apples. Early in the spring of both 1924 and 1925 similar material was studied on the trees in the experimental orchards.

In all cases isolations were made by cutting away the outermost part of the bark with sterile scalpels, using a fresh scalpel for each cut, and then cutting out small pieces of the underlying tissues and treating them by one of the following methods:

(a) The material was ground fine in a sterile mortar in clean sterile quartz sand and sterile distilled water, and this mixture then plated in nutrient dextrose agar.

(b) The material was placed in a sterile petri dish, chopped fine with a sterile scalpel, mixed with ½ cc. of sterile distilled water, allowed to stand for 10 minutes, and then plated as just described.

(c) The material was placed in a tube of nutrient dextrose broth, chopped fine with a sterile scalpel in the neck of the tube, then mixed with the broth and incubated 24 to 48 hours at 28° C.

The pathogenicity of all cultures obtained was tested by pricking broth cultures or sterile distilled water suspensions of the organism with sterile needles into succulent twigs of Wealthy, Fameuse, or Transcendent trees.

From the cut branches sent to the laboratory, platings were made in some cases as soon as they were received, and in others after they had been kept for varying periods with their lower ends immersed in water. The cuts on the lower ends of the branches were made fresh from time to time, those at the upper ends were paraffined to prevent excessive drying, and the water in which the branches were placed was changed daily. The branches were kept in the 70° F. greenhouse until the buds had burst and the leaves had developed as far as they would under such conditions. It was thought that during this time the cankers might become active. Platings were made at various intervals during this period. Some of the branches were connected by means of rubber tubing to a hydrostatic pressure system so that the pressure of a 10-foot water column was applied to the lower cut surfaces. It was thought that this treatment might favor the production of exudate from the cankers. The results of all the above isolation experiments are given in table 1.

In Series III to VI, inclusive, attention was given to the type of margin of the canker, that is, whether it was cracked and well corked off or comparatively smooth and indefinite. It will be noticed that the greater number of isolations of *B. amylovorus* came from the smooth cankers. The organism was isolated in Series II from the live tissues adjacent to the margin of the canker; in Series III from the live tissues adjacent to the margin of the canker and from an area as far as ¾ inch beyond the margin; in Series IV from the margin of the discolored area of a smooth canker; in Series V from the extending margin of a canker on a branch which had been connected with the hydrostatic pressure system for 19 days; in Series VI from the margins and from the tissues up to one inch beyond the margins of smooth cankers. Of 277 cankers examined, 16 (5.7 per cent) were found to be holdover cankers. These figures are in general accord with data obtained by actual counts and observations of cankers in the orchards. Of 352 platings from the margins of cankers, 11 were positive. Of 417 platings from the area ¾ to 1¼ inches beyond the margins of the cankers, 19 were positive. All platings from the dead areas in the centers of the cankers gave negative results.

During the winter 1924–25, in addition to the isolations from cankers sent to the laboratory from the orchards, trials were made from cankers which had been produced upon a pear tree of unknown variety in the pathological garden at Madison by making scalpel incision inoculations into the large branches and trunk on August 13, 1924. Platings made from five of these cankers on February 11, 1925—five trials from the dead tissues in the

TABLE 1.—*Results of attempts to isolate B. amylovorus from apple cankers*

Series and dates of platings	Number and type of cankers[e]	Part of canker from which platings were made					
		Dead center		Margin		Living tissues ¾ to 1¼ inches beyond margin	
		Trials	Results[f]	Trials	Results[f]	Trials	Results[f]
		No.	No.	No.	No.	No.	No.
I[a] Nov. 6 to 28, 1923	25	50	50 –	50	50 –	50	50 –
II[a] Feb. 9 to 26, 1924	27	31	31 –	62	1 +, 61 –	64	64 –
III[b] May 13 to 18, 1924	65 C	65	65 –	75	75 –	75	75 –
	40 S	40	40 –	45	1 +, 44 –	55	2 +, 53 –
IV[c] Nov. 17 to Dec. 8, 1924	7 C	7	7 –	7	7 –	13	13 –
	18 S	18	18 –	18	1 +, 17 –	23	23 –
V[c] Feb. 24 to Mar. 15, 1925	24 C	24	24 –	24	24 –	38	38 –
	11 S	11	11 –	11	1 +, 10 –	23	2 +, 21 –
VI[d] Apr. 27 to 28, 1925	60 S	60	60 –	60	7 +, 53 –	85	15 +, 70 –

[a] Cankers on cut branches of Wealthy from Menomonie. Plated at Madison.
[b] Cankers on Wealthy at Menomonie. Plated at Menomonie.
[c] Cankers on cut branches of Fameuse from Gays Mills. Plated at Madison.
[d] Cankers on Fameuse at Gays Mills. Plated at Gays Mills.
[e] C = Cankers with margins well corked off. S = Cankers with smooth margins.
[f] – = *B. amylovorus* not isolated. + = *B. amylovorus* isolated.

centers of the cankers, seven from the margins, and eight from the tissues ¾ to 1¼ inches beyond the margins—gave two positive results. One of these was from tissues ¾ inch beyond the margin and the other from tissues one inch beyond the margin.

On February 14 a branch, 1½ inches in diameter, bearing two cankers was cut from the above mentioned pear tree and placed with the lower end in water in the greenhouse, where it was kept at 50° F. for 10 days, at 60° F. for a week, and then at 70° F. in an attempt to induce extension of the cankers. Before being brought into the greenhouse the cankers had been carefully outlined with a wax pencil. Under this treatment one of the cankers extended its boundaries. On March 17, as the buds were beginning to open, this canker had extended one inch beyond the lower margin, 1/4 inch above the upper margin, and 1/8 to 3/16 inch beyond the side margins.

TABLE 2.—*Results of experiments on the position of the fireblight bacteria in relation to apple cankers just renewing activity, Gays Mills, Wis., 1925*

Dates of culturing	Cankers tested		Results from cultures from stated locations[a]							
	Variety	No.	Dead center	Margin	Newly discolored area			Zone ¾ to 1¼ inches beyond newly discolored area		
					Outer bark	Inner bark	Outer wood	Outer bark	Inner bark	Outer wood
Apr. 27	Hyslop	2	No. 2 –	No. 2 +	No. 2 +	No. 2 +	No. 2 +	No. 2 +	No. 2 –	No. 2 –
Apr. 28	Transcendent	3	3 –	3 +	3 +	3 +	2 +, 1 –	2 +, 1 –	3 –	3 –
Apr. 29	Transcendent	3	3 –	3 +	3 +	3 +	1 +, 2 –	3 +	1 +, 2 –	3 –
Apr. 30	Hyslop	3	3 –	3 +	3 +	3 +	3 +	3 +	3 +	3 –
May 16	Fameuse	5	5 –	5 +	5 +	5 +	2 +, 3 –	4 +, 1 –	1 +, 4 –	5 –
May 18	Fameuse	5	5 –	5 +	5 +	5 +	4 +, 1 –	3 +, 2 –	2 +, 3 –	5 –

[a] + = *B. amylovorus* isolated. – = *B. amylovorus* not isolated.

Platings were made from the dead central tissues and from these extending margins. No bacterial growth was obtained on the plates made from the dead tissues, but the plates made from the extending margins showed apparently pure cultures of bacteria which were proved by pathogenicity tests on young growing apples trees to be *B. amylovorus.*

The above experiments give further proof that the overwintering of the blight organism occurs, not in the dead tissues of the cankers, but in the apparently healthy tissues adjacent.

The isolations just reported were all made from material collected in a dormant condition. The following tests were made from cankers which had just resumed activity. By using cankers in which the organism was known to be present, and by making a greater number of trials from each canker, an attempt was made to secure more definite information as to the distribution of the organism in relation to the canker. The cultures were made by cutting away the tissues with sterile scalpels until the parts desired were uncovered without contamination, then cutting out a small piece with a sterile scalpel and placing it in a sterile tube of nutrient dextrose broth which was incubated for from 24 to 48 hours. The pathogenicity of these cultures was tested by pricking them into young apple twigs of Fameuse which had previously been protected by paper bags to insure their being free from blight. The results are given in table 2.

Here again the organism was not found in the dead tissues in the centers of the cankers but was isolated from the outer and inner bark of the extending margin in every case, and from the wood beneath the extending margin 13 times out of 21. From the area ¾ to 1¼ inches beyond the extending margin, it was isolated from the outer bark 17 times out of 21, and from the inner bark 7 times out of 21, but not from the wood beneath. These results give a gross conception of the relation of the organism to the host tissues during the progressive development of cankers. In the advancing zone the organism is present in the outer part of the bark, extending well beyond the macroscopically affected area, while in the later stages of development it progresses inward until it reaches the cambium.

Studies of cankers: The percentage of cankers overwintering B. amylovorus. Observations were made with the aim of securing more definite information as to the percentage of cankers which overwinter the blight organism. The results, which appear in table 3, show that the organism overwintered in sufficient abundance to furnish a plentiful source of inoculum for primary infection. The counts made on May 13 showed a much higher percentage of holdover cankers than usual due to the fact that the 100 cankers observed had been selected because of their smooth margins.

Studies of blighted twigs: Whether the blight organism is able, under Wisconsin conditions, to survive the winter in the small blighted twigs

remaining on the trees had not been definitely determined. Reinking (32), working upon this problem at the University of Wisconsin, was unable to isolate *B. amylovorus* from blighted twigs late in the fall, during the winter, or early in the spring. In Colorado, however, Sackett (33) was able to isolate the organism from as high as 21 to 33 per cent of blighted pear twigs. With these results in mind, the writer carried on the following work.

Platings made from blighted twigs brought to Madison from the orchards during the late fall and winter of both 1923 and 1924 all gave negative results, as did also those made in the early spring of 1924 from blighted twigs on Wealthy trees in the Weston Orchards. The platings were made from the basal portion of the dead tissues of the twigs and also from the live tissues immediately adjacent.

TABLE 3.—*The percentage of cankers overwintering B. amylovorus*

Place and date of observation	Variety	Trees No.	Cankers examined No.	Cankers active	
				No.	Per cent
Menomonie, Wis.					
July 22, 1924	Mixed	—	104	8	7.7
July 24, 1924	Wealthy	—	202	5	2.5
Gilys Mills, Wis.					
May 13, 1925	Fameuse	30	100	11	11.0
May 19, 1925	do	35	160	13	8.1
May 21, 1925	do	145	291	16	5.1
do	Wealthy	55	25	1	4.0
May 22, 1925	do	100	129	6	4.6

It was not until the early spring of 1925 that observations in the orchards brought out the possible importance of small blighted twigs as holdovers of blight. On May 7 two McMahon twigs ¼ inch in diameter, which had been blighted the previous year, were found overwintering the organism. Three such twigs, 3/16, 1/4, and 3/8 inch in diameter, respectively, were found on a Hyslop crab; and three twigs, ½, ⅜, and ¼ inch in diameter, respectively, were found on a Transcendent crab. Each of these twigs had a drop of exudate on the live tissues immedately adjacent to the dead area.

On May 8, 9, and 10 several hundred trees and thousands of twigs which had been blighted the year before were examined to find what per-

centage of them overwintered the organism, as indicated by the production of the typical bacterial exudate. The results, which are given in table 4, show that overwintering of the organism in apple twigs may be of more importance than hitherto supposed. It is desirable that similar data be taken over a period of years and in other sections.

Studies of mummified apples. During the winters of 1923–24 and 1924–25 and the early spring of 1924 cultural studies of mummified apples clinging to blighted twigs failed to show the presence of *B. amylovorus* in any instance. The carpels of some of these apples contained a slimy ooze similar to the bacterial exudate produced by the blight organism, but all attempts to isolate the latter from this ooze gave negative results.

PRODUCTION OF BACTERIAL EXUDATE

Exudate is produced, under certain conditions, from all the blighted parts. In some cases exudation takes place even before the disease is macroscopically apparent in the tissues. In order to determine some of the factors influencing the production of exudate from blighted apple twigs, the following experiments were performed.

TABLE 4.—*The percentage of apple twigs blighted in 1924 which showed bacterial exudate, Gays Mills, Wis., May 8–10, 1925*

Variety	Trees	Blighted twigs observed	Blighted twigs bearing bacterial exudate	
	No.	No.	No.	Per cent
McMahon	360	5525	148	2.5
Fameuse	135	4300	28	0.6
Wealthy	250	1850	27	1.5

Eight Wealthy apple trees in 8-inch pots were used for each experiment, two being placed in each of four chambers (12) in which the temperatures were controlled. Two twigs on each tree were inoculated, and the points of inoculation were covered for 48 hours with cheesecloth wicks which dipped into small flasks of distilled water. The trees were placed in the chambers immediately after inoculation. Three sets of these inoculations were made on different dates. The rate of evaporation in the different chambers was the same and indicated relative humidities of 85 to 90 per cent in the 28° C. chamber, 80 to 85 per cent in the 24° C. chamber, 75 to 80 per cent in the 16° C. chamber, and 60 to 65 per cent in the 10° C. chamber. The results, which were taken after suitable incubation periods, are given in table 5.

While these data are too limited to be conclusive, they suggest that the optimum temperature for the production of exudate lies somewhere between 16° C. and 24° C. These results are in general agreement with those secured from the seasonal development studies of blight made in the orchards.

In 1925 the following sets of experiments were carried on in three controlled humidity chambers (22) the temperatures of which were 20° C. and the relative humidities, 50, 80, and 95 per cent, respectively. At the same time the relation of the vegetative vigor of the host plant to the production of exudate was studied.

On January 24, Wealthy trees were set in 8-inch pots, some in the ordinary potting soil and others in a mixture of potting soil and well rotted manure. They were kept in a greenhouse at approximately 60° F. On February 5 the buds started to burst. In order to secure excessively succulent growth some of the trees potted in soil and manure were treated at two week intervals with a nitrate solution.

TABLE 5.—*The relation of temperature to the production of bacterial exudate from blighted apple twigs, Madison, Wis., 1925*

Temperature in degrees C.	Production of exudate on twigs inoculated at stated dates		
	March 23	March 30	April 8
10	Little	Very little	None apparent
16	Abundant	Fairly abundant	Fairly abundant
24	Fairly abundant	Little	Little
28	None apparent	None apparent	None apparent

On March 16, two twigs of each of six trees were inoculated with a culture of *B. amylovorus* and the trees then placed in a moist chamber at 20–22° C. for 24 hours, after which time one of the trees which had received the special fertilizer treatment and one which had not were placed in each of the controlled humidity chambers. From time to time the production of exudate was noted. Final results (Series I) were taken on March 27.

On March 30 a similar experiment was performed. In this instance, however, instead of placing the trees for 24 hours in the moist chamber, the points of inoculation were covered for 48 hours with moist cheesecloth wicks which dipped into small flasks of distilled water. Final results (Series II) were taken on April 6. Results for both series are recorded in table 6.

From these experiments it appears that, although exudation takes place at all three humidities tested, it is most conspicuous at 80 per cent and is

more pronounced on exceedingly succulent twigs than on those of moderate growth. It is possible that at the highest humidity the exudate may have been rendered inconspicuous by taking up sufficient moisture to diffuse it.

In addition to these greenhouse experiments, observations upon exudate production were made in the experimental orchards. Records were kept of the days upon which exudation was observed. This record is given in Fig. 1, where it is correlated with other data. Before each period of exudate production there was a period of moist cloudy weather with moderately high temperature. Humidities above 80 per cent seemed most favorable for the production of exudate. Cloudy days followed by clear weather favored exudation. Probably one of the most important factors in the production of exudate is the condition of growth of the host plant. Just what this relation is has not been definitely determined.

TABLE 6.—*The relation of humidity and the condition of growth of the host plant to the production of bacterial exudate from blighted apple twigs, Madison, Wis., 1925*

Relative humidity in per cent	Production of exudate	
	From twigs with excessive growth	From twigs with ordinary growth
Series I		
50	Abundant	Little
80	Very abundant	Fairly abundant
95	Little	None apparent
Series II		
50	Fairly abundant	Little
80	Abundant	Fairly abundant
95	Little	None apparent

VIABILITY OF THE CAUSAL ORGANISM

Many investigators have worked upon the problem of the viability of *B. amylovorus* under various conditions, either in artificial culture or in naturally infected material. Gossard and Walton (17) found the organism to be viable in aphid honey dew 10 days after inoculation, and in honey for at least 72 hours. Fulton (16) showed that when blighted twigs were cut off during the period from July to late September and allowed to lie on the ground, nine-tenths of the twigs did not contain viable organisms after 7 days.

Under natural conditions *B. amylovorus* has been found by numerous workers to live over the winter in cankers and, in the present work, it has been shown to overwinter in blighted apple twigs even as small as 3/16 inch in diameter. In an endeavor to determine how long the organism remains viable in drops of exudate under varying conditions, the following experiments were performed.

Exudate exposed to direct sunlight. On June 27, 1925, fresh drops of naturally occurring exudate were collected from petioles, leaf-blades, small twigs, and small pieces of bark. These drops were exposed in open petri dishes to direct sunlight outside the field laboratory for varying lengths of time. After each of the different periods of exposure, 25 drops of the exudate were placed in tubes of nutrient dextrose broth, one drop in each tube, and incubated for 24 to 48 hours, at the end of which time the pathogenicity of the resulting cultures was tested on young blight-free Fameuse apple twigs, one twig being used to test each culture.

TABLE 7.—*The viability of B. amylovorus in drops of exudate exposed to direct sunlight, Gays Mills, Wis., 1925*

No. hours exudate exposed to direct sunlight	No. twigs inoculated	No. twigs blighted
½	25	25
1	25	25
3	25	25
6	25	25
9	25	21
15	25	10
22	25	2
30	25	0

The results which appear in table 7 show that the blight organism was killed in drops of exudate exposed to sunlight for a period of 30 hours. On the trees, however, where the exudate is not exposed to direct sunlight, the organism can probably live for a much longer time.

Exudate on cut twigs exposed to direct sunlight for two hours each day. On June 19, 1925, small twigs bearing abundant exudate were collected. After the leaf-blades had been cut off, these twigs were kept in wire baskets so that they were exposed to direct sunlight for only two hours each day.

After intervals of 7, 15, 25, and 35 days, cultures were made, each time from 25 drops of this exudate, and the pathogenicity tested as in the preceding experiment. Positive results were obtained after 7 and 15 days, but not after 25 and 35 days.

Exudate kept in wax paper bags. During July, 1924, large drops of exudate were collected, placed in wax paper bags, and kept in the laboratory. Nine months later the residues from these drops were cultured in broth, and the pathogenicity of these cultures tested. In no case was blight produced.

On May 19 and June 18, 1925, petioles bearing drops of exudate were placed in wax paper bags and pinned on the wall of the field laboratory so that they were open to the air but not in the direct sunlight. From time to time drops of this exudate were cultured and the pathogenicity of these cultures tested. Tests made 7, 20, 31, 40, 49, and 65 days after collection showed that the exudate still contained *B. amylovorus* in a viable condition. Lack of material prevented the tests being carried on for longer periods.

MODES OF DISSEMINATION OF THE CAUSAL ORGANISM

Though much work has been done on the modes of dissemination of *B. amylovorus*, additional studies of certain aspects of dissemination seemed desirable.

Meteoric water. Direct proof that rain is an active agent in the spread of fire-blight was first obtained by Gossard and Walton (17) in a series of experiments which were so controlled that rain was the only possible agency through which blight might be spread to the branches of the apple trees used for these tests. Stevens *et al* (34) conclude that the blight organism may be spread to a marked degree by the wind. Faulwetter (13) has demonstrated the manner in which *Bacterium malvacearum* may be disseminated by means of wind-blown rain, and suggests the possibility of other bacterial diseases of plants being spread in a similar manner (14).

In the present work, the part that meteoric water might play in the spread of blight was studied along two lines: (a) by observations in the orchards and (b) by infection experiments.

Owing to the very light infection of blight in the Weston Orchards during the season of 1924, no data could be secured upon this phase of the problem. However, observations made in the Kickapoo Orchards during the early development of blight in April and May of 1925 brought out the importance of rain as a factor in the spread of the disease. Even before much pink of the petals had been exposed in the blossom clusters, considerable blight had appeared on the young growing shoots, which at that time were from ½ to 1½ inches long. Upon close observation it was found that these infected shoots formed cone-shaped areas in the trees, and at the apices

of these cones were found actively exuding holdover cankers or twigs. Until about May 13, these infection cones could easily be traced to their sources of inoculum. Later they were partially obscured by the development of blossom blight. However, even some of the early blossom blight was found to be due to inoculum which was carried down by rain from exuding twigs above the blossom clusters.

Experiments were performed in order to determine whether infection from rain-borne inoculum could take place and under what conditions it would occur. The results indicate that rain can, under certain conditions, play an important part in the spread of blight. Dissemination of the blight organism over the individual tree, and to a limited extent from tree to tree, can be brought about by rain or heavy dews.

Insects. Ever since Waite (41) showed the relation of the honey bee to the spread of blossom blight, and Stewart (36, 37, 38) found that twig blight is spread through the agency of sucking insects, much interest has

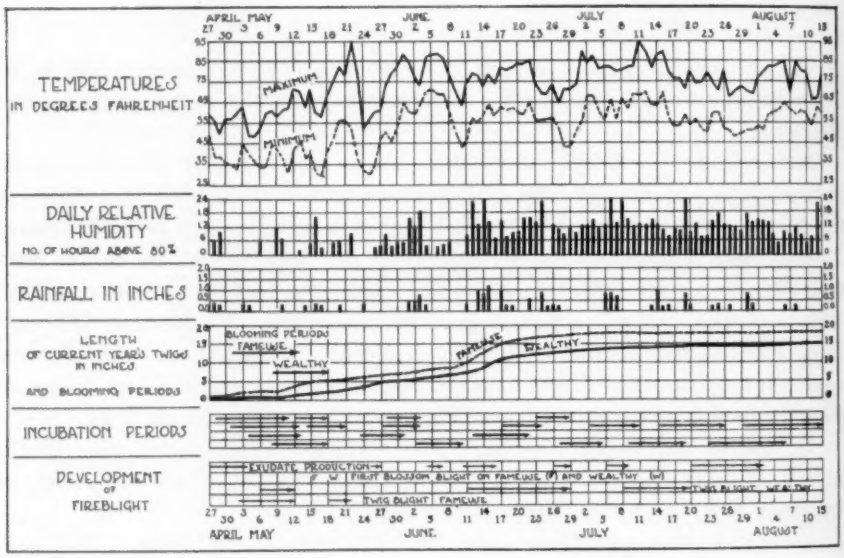

FIG. I.—Graphic summary of seasonal development and meteorological data, Gays Mills, Wis., 1925.

The meteorological data were taken in the experimental orchards by means of a hygrothermograph housed in a standard instrument shelter and a rain and snow guage of the type used by the U. S. Weather Bureau.

The length of current year's twigs is based on averages of records taken on fifty terminal shoots of each variety, representatively located on several trees.

The records on length of incubation period are based on puncture inoculations of current year's twigs of Fameuse and Wealthy.

been attached to insects as disseminators of fireblight. The list of offenders is a long one. Burrill (4) proved that aphids and leaf hoppers spread twig blight. B. J. Jones (23) listed many blight-disseminating insects, from flies to click beetles. D. H. Jones (25) showed the importance of the fruit-bark-boring beetle. Merrill (27, 28) further demonstrated that aphids are responsible for the spread of fireblight.

During the present work attention was given only to those insects which appeared in large numbers in the experimental orchards. In addition to the bees, the other important insects in disseminating blight were grain aphids, apple aphids, and leaf hoppers. In 1925 experimental and observational work was carried on with these two species of aphids and observations were made upon leaf hoppers to determine the extent to which these insects are responsible for the spread of twig blight. The results of this work, which appear below, accord with those reported by Burrill (4).

During the last week in April, 1925, the grain aphid, *Aphis avenae* Fab., was plentiful upon the apple trees. They not only covered the tips of the young growing shoots, but also travelled from place to place in the trees, crawling up and down the large branches and also upon the trunks. In their wanderings they traveled over exuding cankers and were observed many times to be caught in the bacterial exudate. In several instances the aphids were seen at the edge of the drops of exudate feeding upon it. It was noticed that the aphids feeding upon the drops of exudate remained there for several hours, but finally left it and went to other parts of the trees. Thus it is readily possible that aphids becoming contaminated in this way may bring about initial infection of spurs and twigs in the early spring. Examination of some of the blossoms just before they opened showed that aphids were present not only on the outside of the petals, calyces, and pedicels, but also inside the blossoms on the petals, filaments, styles, stigmata, and in the nectar. Since the aphids were found on exuding cankers and in the nectar of blossoms, it is possible that some of the initial infection of blossoms is brought about as a result of aphid-borne inoculum even before the blossoms are fully opened.

The results of the following experiment further strengthen the evidence that *A. avenae* can spread blight. On May 8, 10 young twigs of a Fameuse tree the tips of which were covered with *A. avenae* were inoculated with a suspension of *B. amylovorus* by means of a fine hypodermic needle. The inoculations were made about one inch below the tips at a point below the lowest feeding aphid. When the first symptoms of blight appeared, the twigs were cut off and the aphids transferred by means of camel-hair brushes to healthy twigs which had been enclosed in parchment paper bags for a sufficient period to ensure their being free from blight. Forty aphids were transferred from each of the ten twigs to healthy twigs, twenty aphids

to each twig. These healthy twigs bearing the aphids were then enclosed in celluloid cylinders as a protection against natural infection and also to protect the aphids against predatory insects, and were observed every day. Within 14 days all 20 of the twigs had blighted. As the twigs upon which the aphids are feeding become wilted from blight, the aphids commonly leave them and go to other young healthy shoots. This habit favors dissemination of twig blight.

An experiment similar to the above was performed on June 4. Five water sprouts of a Hyslop tree, the tips of which were covered with *Aphis pomi* De Geer, were inoculated by means of a hypodermic needle with a suspension of the blight organism as in the preceding experiment. As soon as the first symptoms of blight appeared, the twigs were cut off and the aphids transferred to healthy, blight-free water sprouts on Wealthy apple trees. These sprouts were enclosed in celluloid cylinders, and by June 20 all of them were blighted.

From the middle of June, 1925, to the middle of July, leaf hoppers were abundant on the apple trees. They caused the characteristic hopper injury to the leaves: the curling under and yellowing of the edges and a light brown specking of the under sides of the veins due to their feeding punctures. As blight appeared during this period, it was noticed in many cases that the disease first developed in the midribs of the leaves and from there proceeded down through the petiole into the twigs. From these observations it would appear that leaf hoppers are either capable of directly spreading blight or of so puncturing the leaves that infection may take place by means of rain-borne inoculum.

In Wisconsin, aphids and leaf hoppers appear to be the more important insects in the spread of twig blight. Click beetles do not occur in sufficiently large numbers to make them the menace they seem to be in California orchards (23) or in Oregon (8). The tarnished plant bug does not seem to be as plentiful in Wisconsin as it is in New York (36).

INFECTION STUDIES

Mode of entrance of causal organism. It is generally accepted that fire-blight infection of apple may take place through the nectaries of blossoms, but whether or not infection can take place through other natural openings, such as stomata and hydathodes of leaves, is still an open question. It was earlier thought that the blight organism gained entrance into leaves and twigs only through wounds, but in 1915 Heald (19) reported that probably stomatal and hydathodal infection takes place. In order to determine whether or not stomatal infection occurs under Wisconsin conditions, the following experiments were conducted.

The experiments of 1924 were performed in the orchard upon young Wealthy apple twigs which were apparently uninjured, although no attempt had been made to protect them previous to the time of inoculation. It was realized that, in this case, negative results as to stomatal infection would be valid, while positive results would not necessarily prove that stomatal infection had occurred, because there may have been microscopic injuries in twigs and leaves used. On June 30, a cloudy day, the upper and lower surfaces of the leaves on 30 young growing twigs were sprayed with a 48-hour-old broth culture of *B. amylovorus* diluted one-half with sterile distilled water. Ten of the twigs were enclosed in celluloid cylinders while the others were left exposed. By July 8 the apical leaf on each of five of the exposed twigs was blighted, but was not spotted as might be expected had stomatal infection taken place. When the final results were taken on July 23 no other twigs had developed blight.

On the evening of July 3 the leaves on 20 twigs similar to the above were inoculated in the manner just described, and none of them were enclosed. On July 12 the only blight evident was on the apical leaf of one twig.

Early in the morning of July 10, while wet with dew, the leaves of 40 twigs were inoculated as described above, and all the twigs were enclosed in heavy manila paper bags. The leaves of the twigs used had all expanded, but the terminal leaves were still immature. By July 23 none of these twigs had blighted.

During the winter of 1924–25 experiments were performed in the greenhouses in which potted trees were subjected to various environmental conditions before and after spraying the leaves with suspensions of the blight organism in an attempt to secure stomatal infection.

On March 9 the upper and lower surfaces of the leaves of four twigs on each of eight Wealthy trees which had been kept at approximately 70° F. were sprayed with a suspension of *B. amylovorus*. After inoculation the trees were kept in a moist chamber at 65–75° F. for 24 hours. They were then placed in the controlled temperature houses, two trees at each of the following temperatures: 50, 60, 70, and 80° F. The trees in the 70° and 80° F. houses did not develop blight. The tip of the youngest leaf on each of two twigs on trees kept at 50° F. and the youngest leaf on one twig of a tree kept at 60° F. became browned, and at 50° F. exudate formed on the petioles of the discolored leaves. In no case, however, was there definite indication of stomatal infection. The small percentage of infection which did take place may well have been due to microscopic wounds in the young leaves.

On March 16 a similar experiment was performed, using the leaves on two twigs of each of four trees. After inoculation and 24 hours in the the

moist chamber at 67° F., two trees were placed in the 60° F. house and two in the 70° F. house. None of the twigs developed blight.

In the next two experiments the trees were placed in the moist chamber at 65–75° F. for 36 hours before inoculation and for 24 hours after inoculation, in an attempt to have the stomata wide open and the substomatal cavities and intercellular spaces filled with moisture.

On March 11 three small Bartlett pear trees were taken from the moist chamber, and the upper and lower surfaces of the leaves of four twigs on each tree were sprayed with a suspension of *B. amylovorus*. The trees were replaced in the moist chamber for 24 hours, and then one tree was placed in each of the controlled temperature houses which ran at 60, 70, and 80° F., respectively. After five days the tips of the twigs on the trees kept at 60 and 70° F. showed slight browning, which later worked downward until the entire twigs were blighted. At 80° F. blighting did not occur so rapidly.

On March 11 an experiment parallel to the previous one was performed, using six Wealthy apple trees. The leaves on two twigs of each tree were inoculated and treated as described above, and two of the trees were placed in each of the controlled temperature houses at 50, 60, and 70° F. None of the leaves developed blight.

During the next growing season these experiments were resumed in the experimental orchard. The varieties used were Fameuse and Wealthy. Broth cultures of *B. amylovorus* diluted with water were sprayed only on the leaves of young twigs. Inoculations were made early in the morning, late in the evening, or on cloudy days, in the hope of catching the stomata when they were wide open. Before the time of inoculation about 50 per cent of the twigs used had been protected from natural infection by parchment paper bags. This method of protection was not always satisfactory because of the browning which took place at points where the leaves touched the paper. Consequently, half of the twigs chosen for the experiments had been unprotected previous to inoculation. They were, however, twigs which had grown rapidly during a rainy period in June and were apparently comparatively free from injury. Inoculations were made on May 20, 23, 27, 29, and June 2, 4, 12, and 15. After inoculations some of the twigs were enclosed in celluloid cylinders or paper bags, while some were left exposed. A summary of the results of these experiments is given in table 8.

None of the twigs which were protected from natural infection blighted; and of those not protected, eight twigs became completely blighted and seven had merely the tip leaves blighted. In no case was there evidence of stomatal infection.

In the above experiments the entire surfaces of the leaves were sprayed with the suspensions of the organism, but in the following tests the inoculum

was applied to small areas of the leaf surface in an attempt to minimize possible confusion incident to penetration of the organism through wounds. Circles ½ inch in diameter were painted on the lower surfaces of leaves with India ink and a camel-hair brush. A drop of the suspension of *B. amylovorus* was applied to the center of each circle by means of a camel-hair brush. Four or five of the youngest leaves on each twig were used. The inoculations were made on June 11, a cloudy day followed by another cloudy day with high humidity and two inches of rainfall. The twigs were observed daily but no blight developed. In view of the negative results of these and the preceding experiments it seems safe to conclude that under Wisconsin conditions stomatal infection does not commonly occur.

TABLE 8.—*Results of experiments on stomatal infection of apple leaves by B. amylovorus, Gays Mills, Wis., 1925*

Variety	Results on twigs which received stated treaments after inoculation					
	In celluloid cylinders		In paper bags		Exposed	
	No. inoculated	No. blighted	No. inoculated	No. blighted	No. inoculated	No. blighted
Fameuse	26	0	28	0	68	7
Wealthy	22	0	9	0	40	8

If stomatal infection does not take place, then to what extent must a leaf or stem be injured in order that rain-borne blight organisms may gain entrance and produce the disease, and for how long a time do these wounds remain active as infection courts?

In the Weston Orchards during the 1924 season it was observed that many of the leaves on the trees were injured by strong winds. There was the possibility that these injuries served as avenues of entrance for rain-borne inoculum. The following experiments were performed in an attempt to determine to what extent this was true.

Late in the afternoon of June 30, thirty twigs with naturally injured leaves were sprayed with a suspension of *B. amylovorus* and then covered with paper bags. By July 11 three of these twigs were blighted.

A similar inoculation of the leaves of 30 other twigs was made on July 11. The twigs were enclosed in paper bags after inoculation. Results taken July 26 showed that blight had developed in 10 of the twigs. These two experiments show that the blight organism may gain entrance into twigs through the wounds in leaves.

On July 6 the leaves on 30 twigs were stabbed with sterile scalpels so that the wounds were made through the intervenal areas and also through

some of the larger lateral veins. Immediately after wounding, the leaves were sprayed with a suspension of *B. amylovorus* and then covered with paper bags. Results taken July 18 showed that 27 of the twigs had blighted. From this experiment it was evident that fresh wounds on leaves may serve excellently as avenues of entrance for the blight organism. The next experiments were conducted to determine how long wounds serve as infection courts.

During the winter of 1924–25 experiments were conducted in the greenhouse. On March 10 the leaves of three twigs on each of six Transcendent trees were slit with sterile scalpels, cutting through the lateral veins. At intervals of 0, 2, 4, 8, 16, 24, 36, and 48 hours after injury, the leaves of two twigs were sprayed with suspensions of the blight organism. Each time the unsprayed twigs were carefully protected from the spray. After inoculation the twigs were enclosed in wax paper bags containing small wads of moist cotton. The results showed that blighting occurred in the twigs inoculated 0, 2, 4, 8, 16, 24, and 36 hours after injury, but not in the twigs inoculated 48 hours after injury.

On March 15 a similar experiment was performed, the inoculations being made 36, 48, 60, and 72 hours after injury to the leaves. Blight developed in the twigs inoculated 36 hours after injury, but not in those inoculated 48, 60, and 72 hours after injury.

On March 17 an experiment similar to the above was performed, using Wealthy apple trees. The results likewise showed that, up to 36 hours after injury to the leaves, the wounds served as infection courts for the fire-blight organism, but at the end of 48 and 60 hours these wounds were so changed that the organism did not gain entrance.

During May, two sets of the same type of experiment as the above were conducted in the orchard on Fameuse trees. The results showed that, after 30 hours, the wounds in the leaves no longer served as avenues of entrance for the blight organisms.

In the above experiments the larger veins of the leaves were injured, but in the following experiments the wounds were so made with the point of a scalpel or needle that none of the larger veins were injured.

On July 13, 1924, the laminas of the leaves on 30 twigs on Wealthy trees were punctured by needles so that none of the veins were injured. Immediately after injury the leaves were sprayed with a suspension of *B. amylovorus* and then enclosed in paper bags. By July 29 only one of the twigs was blighted.

On May 20, 1925, a similar experiment was performed, using the leaves on 25 twigs of Fameuse. No blight developed in any of the twigs.

Two further sets of this same type of experiment were conducted during June, 1925, upon the leaves of 25 twigs of Wealthy and 17 twigs of Fameuse.

Blight did not develop in the Wealthy twigs but did develop in one twig of Fameuse. This latter case may have been due to natural infection, since the Fameuse twigs were not protected previous to inoculation.

The results of the foregoing experiments tend to show that infection of leaves takes place more readily, if not exclusively, through wounds in the veins, and to a less extent, if at all, through wounds in the intervenal areas. Furthermore, these wounds in the leaves soon become altered in some way, possibly merely by drying out, so that they no longer serve as infection courts for *B. amylovorus*. In nature, of course, leaf infection very commonly results from passage of the blight organism from the subtending twig through the petiole.

Studies similar to the above were made upon injuries to stems of growing shoots. On July 14, 1924, the stems of 15 growing shoots of Wealthy apples were injured by pricking with fine sterile needles and immediately after injury were sprayed with a suspension of the blight organism and then enclosed in paper bags. By July 31 all of the twigs had blighted.

On May 27, 1925, the stems of 84 shoots of Fameuse were punctured each 20 times with fine sterile needles. At intervals of 0, 2, 4, 8, 19, 30, and 52 hours after injury, 12 of the stems were sprayed with a suspension of the blight organism. By July 16 all 84 of the twigs had blighted.

On June 4, 1925, the stems of 72 shoots of Fameuse were injured as described above; and at intervals of 48, 60, 72, and 84 hours after injury, 18 of the stems were sprayed with suspensions of *B. amylovorus*. By June 22 the twigs inoculated 48, 60, and 72 hours after injury had developed blight, but those inoculated 84 hours after injury had not.

On June 12 the stems of 100 twigs of Fameuse were punctured as in the preceding experiment; and at intervals of 24, 34, 44, 54, and 74 hours after injury, 20 of the twigs were sprayed with a suspension of the blight organism. By July 8 most of the twigs had developed blight, except those inoculated 74 hours after injury, of which only three had blighted.

The results of these experiments upon stem injury show that the wounds in the stems serve as points of entrance for the fireblight organism, but within approximately 74 hours after injury the wounds become so altered that they no longer serve as infection courts.

Factors influencing infection. That different varieties of apple vary in their resistance to fireblight has been observed by other workers. Among those who have published lists of varieties classified according to the susceptibility or resistance to blight are Anderson (2), Cate (8), Chambers (9), Green *et al.* (18), Hedrick and Howe (20), Hewitt (21), Norton (29) Stewart (35), Swingle (39), and Whetzel (43).

During the two year period, 1923–25, the relative susceptibility or resistance to fireblight of the different varieties of apple grown on a commercial

scale in Wisconsin was observed. In the following list these varieties are arranged according to their apparent susceptibility to blight under the conditions observed.

I. Very susceptible: Hyslop, Transcendent, Yellow Transparent, McMahon, Fameuse, and Wealthy.

II. Moderately susceptible: Tolman Sweet, Black Ben Davis, Jonathan, and Salome.

III. Moderately resistant: McIntosh, Duchess of Oldenburg, Grimes, Orange Winter, Wolf River, Whitney, and Stayman Winesap.

IV. Resistant: Ben Davis, Charlemoff, Delicious, Dudley, Northwestern Greening, and Tetofsky.

The Hyslop and Transcendent were the worst blighters observed. Large body cankers were found on trees of these varieties. Wealthy, one of the most widely planted varieties in Wisconsin, is very susceptible to twig blight, as is also the Fameuse. Dudley, in most cases, appeared to be fairly resistant. McIntosh had a small amount of twig blight which did not run down into the larger branches. Some spur blight resulting in the formation of small cankers appeared on the Duchess, but practically no twig blight or overwintering of the organism occurred on this variety. Delicious, Northwestern Greening, and Tetofsky seemed to be the most resistant of the varieties observed.

The exact nature of resistance of plants to fireblight has not been defined. It has, however, long been a matter of observation and record that plants in a high state of vegetative vigor are more susceptible to this disease than

TABLE 9.—*The relation of humidity and the condition of growth of the host plant to fireblight development, Madison, Wis., 1925*

Relative humidity in per cent	Development of blight			
	In twigs with excessive growth		In twigs with ordinary growth	
	Average length of blighted area	Average daily increase in length of blighted area	Average length of blighted area	Average daily increase in length of blighted area
Series I	Inches	Inches	Inches	Inches
50	5	0.42	2	0.18
80	11	1.00	5	0.42
95	9	0.82	4	0.36
Series II				
50	2	0.29	0.5	0.07
80	4	0.57	1.3	0.19
95	3	0.43	1.0	0.14

are those in low vigor. Greenhouse experiments substantiate these observations. Potted plants which had been forced into a vigorous, succulent growth blighted much more severely than did unfertilized trees which were in a lower state of vegetative vigor (Table 9).

In order to determine what influence temperature and humidity have upon the development of blight, the following experiments were performed. The methods of procedure with the controlled temperature experiments and those for the controlled humidity experiments have been explained above. From the results, which are given in table 9, it appears that blight develops more rapidly at the higher humidities, 80 per cent being slightly more favorable than 95 per cent. It also appears that the trees which had excessive succulent growth developed much more blight than did those which had not received the special fertilization treatment.

Series I of the temperature experiments reported in table 10 was conducted in the large controlled temperature greenhouses. The temperatures varied as much as two degrees from those given in the table. Series II was conducted in the small controlled temperature chambers where the temperatures did not vary more than one degree from those given. From these results it appears that the optimum temperature for the development of fireblight lies somewhere between 21° and 28° C. (70° and 82° F.).

SEASONAL DEVELOPMENT STUDIES

A summary of the records pertaining to the development of fireblight and of the apple plant for the season 1925 is given in figure 1, where they are correlated with certain meteorological data.

It will be observed that periods of exudate production were more dependent upon external environmental factors in the latter part of the season than they were early in the season. It may be that during the early part of the season the condition of the host is such that exudation will take place under a much wider range of external conditions than is the case later, when periods of high humidity, fairly high temperatures, cloudy days, and sufficient rainfall are necessary.

In 1925 the blighting of shoots occurred even before the appearance of blossom blight. In May, Fameuse had more twig blight than did Wealthy, but by the middle of June the situation was reversed, twig blight in the Fameuse being of small consequence while in the Wealthy it had occurred in epidemic form. This condition may be partly explained by the fact that the Wealthy had a much heavier bloom than did the Fameuse, and consequently, blight was more widely disseminated through the Wealthy blossoms which then served as foci for further infection of the rapidly growing twigs. From July 16 to August 4 there was little extension of blight in the twigs and but little new infection. After August 4 fireblight was apparently inactive.

TABLE 10.—*The relation of temperature to fireblight development, Madison, Wis., 1925*

Series I			
Date of inoculation	Temperature in degrees C.	Incubation period	Average daily increase in length of blighted area
		Days	Inches
Feb. 24	10	12	0.25
	16	8	0.37
	21	4	0.75
	27	4	0.63
Mar. 8	10	8	0.33
	16	6	0.50
	21	3	1.00
	27	3	1.13
Mar. 5	10	10	0.13
	16	5	0.17
	21	3	0.33
	27	2	0.50

Series II			
Date of inoculation	Temperature in degrees C.	Incubation period	Average daily increase in length of blighted area
		Days	Inches
Mar. 23	10	10	0.25
	16	6	0.66
	24	4	1.25
	28	2	1.13
Mar. 30	10	12	0.13
	16	7	0.50
	24	4	0.63
	28	4	0.63
Apr. 8	10	11	0.07
	16	5	0.38
	24	3	0.88
	28	3	0.75

Macroscopic symptoms of blossom blight did not appear until 13 days after the beginning of the blossoming period. The interval between the appearance of blossom blight on the Fameuse and on the Wealthy was about equal to the interval between the start of the blooming periods of the two varieties.

Artificial inoculations were made at frequent intervals to determine the incubation periods for twig blight throughout the season. The incubation periods of the earlier infections averaged about 13 days. As the season progressed they gradually shortened to a minimum of about 5 days during June, and then lengthened to a maximum of about 15 days in early August. This process, however, was not regular; there was considerable variation in the period of incubation without relation to the stage of the season. Incubation was hastened during periods of high humidity, rainfall, and fairly high temperature.

DEVELOPMENT OF EPIDEMICS

In Wisconsin, fireblight appears to some extent each year and frequently in epidemic form. In a given season it may be mild in its attack in one locality and severe in another. The direct loss to the apple growers is great only during epidemic outbreaks. It is important, therefore, to define as clearly as possible the chief factors which influence the development of epidemics. From the results of other investigators and those herein reported it appears that most of these factors center about weather conditions, disseminators of the disease, and the variety and condition of the host plant.

The present work has shown that the disease develops best at temperatures of about 65° to 85° F., and that epidemics are favored when such temperatures are accompanied by rains or high humidity. Rain, in addition to increasing the susceptibility of the plants by causing succulent growth, is also a direct disseminator of blight.

Since certain insects play a very important role in the spread of fireblight, the more numerous they are in the orchard the greater the chances for a blight epidemic. While the honey bee is perhaps the most important disseminator of blight, its work as a pollinating insect is indispensable in most apple orchards, since most commercial varieties of apple are self-sterile. The sucking insects, in addition to being able directly to transfer the blight organism from infected to healthy apple tissues, also wound the young growing parts so that the rain-borne inoculum can gain entrance.

Serious outbreaks of fireblight have frequently been occasioned through spread of the organism by careless use of pruning tools, or by inducing excessive growth of the host plant by injudicious pruning.

The susceptibility or resistance of the variety of apple has much to do with the overwintering of the blight organism and the subsequent develop-

ment of epidemics. Within a variety the condition of the host plant has an important influence on the extent to which blight will develop. Succulent growth is readily blighted, whereas a woodier growth is less easily invaded. Furthermore, from observations made over a period of three years, it appears that in Wisconsin epidemic outbreaks usually occur during the years of heavy bloom. The heavier the bloom, the greater are the chances for blossom blight to occur.

STUDIES OF METHODS OF CONTROL

Experiments on control measures were performed in the orchards along two lines: (a) the removal or treatment of blighted parts and (b) spraying for the control of insects which disseminate the disease. In 1924 the work was carried on in the Weston Orchards upon Wealthy, and in 1925 in the Kickapoo Orchards upon Wealthy and Fameuse. The trees were from 9 to 13 years old. The experimental plots contained from 75 to 300 trees each. Adequate check plots were left at various points in the orchards. These controls received no special treatment other than the regular lime-sulfur and lead arsenate sprays which were applied to the entire orchard for the control of apple scab.

Excision of blighted parts over small areas. During the first week in April, 1924, all of the blighted twigs and cankers were excised from 75 Wealthy apple trees in the Weston Orchards. The cuts were made 8 to 12 inches below the lowest evidence of blight. The tools and wounds were disinfected with Reimer's solution[2] and the larger wounds were covered with tree paint. When blight developed in the orchard during June, this plot showed very little disease; whereas in the check plots the blighting, though moderate, was about sixteen times as abundant. The following year, when blight appeared in epidemic form, this plot was practically free from the disease. The value of these data, however, is limited by the unusual sparseness of blight throughout the orchard in 1924. Furthermore, the apparently beneficial results noted in 1925 may have been due not only to the removal of blighted material but also to the fact that the trees in the plot thus treated did not bloom very heavily the second year.

During the first week in May, 1925, all blighted twigs and cankers were removed from a plot of 90 trees of Wealthy and Fameuse in the Kickapoo Orchards. On May 25, after blight had developed in that part of the orchard, a count showed that the disease had developed in five trees in the plot where excision was practiced, as compared with ten trees in the adjacent check plot. Later, however, after blossom blight had had a chance to appear, the amount of blight in the two plots was practically the same.

[2] One gram of mercuric chloride and one gram of mercuric cyanide in 1,000 cubic centimeters of water, as recommended through correspondence by Prof. F. C. Reimer.

This tends to indicate that the cutting out of blight in small areas may be effective in reducing the amount of the disease early in the sesaon, but that, with the opening of blossoms, blight may become so widely spread throughcut the orchard by pollinating insects that the difference is obliterated.

Since it was found that many of the small blighted twigs may overwinter the organism and in some instances serve as more important sources of inoculum for initial infection than the larger cankers, on one plot only the blighted twigs were cut out, the cankers being left undisturbed. Observation, later, showed that activity of the organism had been resumed before the twigs were removed, and, as a result, infection had taken place in the twigs below. Consequently the results of this test were invalidated. This observation emphasizes the importance of early action when excision is employed. The work should be done either in the fall or well in advance of the opening of the apple buds.

Treatment of active cankers. In the spring of 1925, when an abundant supply of active cankers was available, different methods of treatment were applied to them. A sufficient number were left untreated to serve as checks. Notes were read only at times when the check cankers were actively exuding.

An aqueous solution of zinc chloride (20 per cent by weight) was painted over 25 cankers without removing the epidermis. This was twice the concentration suggested by Day (11). Three of these treated cankers were still active when examined one month later. One of the cankers of this series showed a clear amber exudate which tested negatively for the presence of *B. amylovorus.* These 25 cankers were on branches 1½ to 3 inches in diameter. In 13 cases, that part of the branch beyond the canker died after treatment.

In 25 cases the epidermis was scraped off with a sharp knife, not only from the cankers but also from the bark 3 to 4 inches beyond their margins. The wounds were painted with the following solution: 1 gm. mercuric chloride, 1 gm. mercuric cyanide, 500 cc. glycerine, 250 cc. 95 per cent alcohol, and 250 cc. water. Two cankers showed activity after treatment, due to the treatment not having included enough of the apparently healthy bark adjacent to the cankers. In only five cases did the part of the branch beyond the canker die under this treatment.

The epidermis was removed from 10 cankers including 8 inches above and below the cankers and 2 inches on each side. The wounds were painted with a mixture of carbol-fuchsin and gentian violet which, according to a report of a paper by Churchman (1), inhibits the reproduction of bacteria. None of the cankers so treated became active, and in only one case did the part of the branch beyond the canker die after treatment.

Briefly, the advantages of the last two methods reported above are that the activity of the bacteria was checked and but little time was required to

treat the cankers. The disadvantage is that much plant surface is left exposed to drying and to bark- and wood-rotting fungi. More work will have to be done upon these methods before they can be recommended for orchard practice.

Insect control. In the experimental orchards the chewing insects were combatted, as a part of the standard practice for the entire orchard, by the use of lead arsenate in the lime-sulfur sprays for scab.

On May 3, 1924, as the buds were bursting, three plots of 250 Wealthy trees each were treated with a miscible oil spray of a concentration of 1 gallon to 15 gallons of water. Good control of aphids was secured, and up to July 5 no blight had appeared on these plots. After that date a slight amount of blight appeared, carried in, most probably, by leaf hoppers from infected trees in the adjacent plots.

To control aphids further, nicotine sulfate spray (1 pint of a commercial nicotine sulphate solution to 100 gallons of water) containing soap or calcium caseinate as a spreader was applied to other plots as soon as the weather was warm enough to make the spray effective. On May 26, 1924, this spray was applied to 600 Wealthy trees, but the amount of blight in this plot in early June was about the same as that in the check plots. The spray had been applied too late to affect early blight infection.

The following year the aphids hatched out early in April (April 7–15), and a nicotine sulfate spray of the same concentration as in 1924 was applied to two plots of 240 trees each (120 Fameuse and 120 Wealthy) on April 14, when the temperature was well above 70° F. Another application of the spray was necessary on May 13, at which time the temperature reached 72° F. On May 25 there was an average of 0.7 blighted twigs per tree in the sprayed plots as compared with an average of 2.2 blighted twigs per tree in the check plots. Later, after blossom blight had appeared, the amount of blight in the sprayed and unsprayed plots was about the same.

From these results it appears that control of aphids reduces the chances of blight development early in the season before blossoming time. When there is a light bloom on the trees, control of aphids would probably do much to control blight in a given orchard even though neighboring orchards were infected. If the bloom is heavy, however, a blight epidemic may be started by the inoculum brought in from neighboring sources by pollinating insects. In such cases it seems necessary, if practicable, to remove the sources of inoculum from large areas.

In view of the fact that the greatest dissemination of blossom blight commonly occurs during the latter part of the blooming period, it was thought worth while to try to limit the spread of this phase of the disease through the use of bee-repellent sprays after sufficient pollination had been accomplished. Preliminary tests, however, showed that the material se-

lected for this purpose, a commercial repellent, failed to repel bees for more than a few minutes.

The present status of knowledge of fireblight does not appear to warrant confident recommendations of a satisfactory, practical method for the control of this disease under such climatic, cultural, and economic conditions as exist in Wisconsin. The most promising lines of attack appear to be timely excision of the blighted parts, insect control, and care in the selection of varieties and in cultural practice. The extent to which each of these methods is practical will vary with local conditions and remains to be determined by further investigation.

SUMMARY

Overwintering of *B. amylovorus* on apple in Wisconsin was found to occur in association with blighted twigs and cankers in the apparently healthy tissues adjacent to the dead areas. The organism was isolated from the tissues as far as one inch from the margins of dormant cankers. From 2.5 to 11 per cent of the cankers and 0.6 to 2.5 per cent of the blighted twigs studied in the spring of 1925 overwintered the organism. The size of twig does not limit overwintering since overwintering was found to occur in twigs as small as 3/16 inch in diameter. Hyslop, Transcendent, Yellow Transparent, McMahon, Fameuse, and Wealthy are the more important of the Wisconsin commercial varieties of apple in overwintering blight.

Most abundant production of bacterial exudate from blighted parts took place at temperatures between 65 and 85° F. and at relative humidities above 80 per cent. Alternate cloudy and sunny periods favored exudation.

B. amylovorus, when exposed in small drops of bacterial exudate to direct sunlight, was killed after an exposure of 22 hours. In drops of exudate kept in the laboratory in wax paper bags open to the air but protected from direct sunlight, the organism remained viable for more than two months and less than nine. In small blighted twigs cut from the trees and exposed to direct sunlight for two hours each day during June and July, the organism remained viable for 15 days but was dead at the end of 25 days.

Fully 90 per cent of the twig infection early in the season of 1925 could be traced directly to water-borne inoculum from exuding holdover cankers and twigs. Rain plays an important part in the local spread of blight throughout the season.

Aphis avenae, A. pomi, and *Empoasca mali* were observed to be the chief spreaders of twig blight in the experimental orchards. Aphids have been found feeding upon drops of exudate and also in the nectar of blossoms and may therefore be a factor in the initial infection of blossoms.

The nectaries of blossoms, and possibly the hydathodes of very young leaves, seem to be the only natural openings through which blight infection of apple takes place. All attempts to secure stomatal infection failed.

Wounds furnish the ordinary avenue of entrance to the bacteria for leaf and twig infection. Fresh wounds in the veins of young leaves serve as infection courts for water-borne inoculum. However, after some hours (36 to 48) these wounds no longer serve as points of entry for the blight organism. The same holds true for the wounds in stems except that these wounds may remain infection courts for as long as 72 hours. Pruning wounds on twigs may serve as avenues of entrance for *B. amylovorus* even during cold weather.

Of the varieties of apple grown on a commercial scale in Wisconsin, those more susceptible to blight are Yellow Transparent, McMahon, Fameuse, Wealthy, Transcendent, and Hyslop. Those showing a fair degree of resistance are Dudley, Duchess of Oldenburg, McIntosh, and Northwestern Greening.

The state of vegetative vigor of the host is a factor in determining the rapidity and extent to which blight develops. Even the more resistant varieties of apple will blight severely if too heavily fertilized and cultivated so as to produce an abnormal, succulent growth. It has also been noticed that blight epidemics tend to appear during the alternate years of heavy blossoming of the trees.

Factors favoring the development of epidemics of fireblight are temperatures from 65 to 85° F., high humidities, cloudy weather, frequent showers, infestations of aphids and leaf hoppers, an abundance of bees and other pollinating insects, the presence of large numbers of trees of the more susceptible varieties, heavy blossoming of the trees, and vigorous vegetative growth.

In the experimental plots the cutting out of blighted twigs and cankers over small areas during the dormant condition of the trees reduced the amount of blight developing early in the season; but, later, blossom blight was spread throughout these plots by pollinating insects which carried the disease in from surrounding infected plots.

Treatment of active cankers by merely painting them with a 20 per cent solution of zinc chloride was not satisfactory in checking their activity. The scraping of the epidermis from active cankers and adjacent bark and the application to the wound of Reimer's solution with the addition of glycerine and alcohol gave comparatively good results. The application of a mixture of carbol-fuchsin and gentian violet to active cankers from which the epidermis had been scraped checked the activity of the organism.

Spraying for the control of certain insect disseminators of blight checked the spread of the disease early in the season. These beneficial results disappeared, however, following the spread of blossom blight by pollinating insects. An attempt to mitigate this difficulty by using a bee-repellent spray after sufficient pollination had occurred was unsuccessful.

Further studies of fireblight are in progress at the Wisconsin Agricultural Experiment Station.

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THE INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON WHITE ROT OF ALLIUM

J. C. WALKER

In a previous report (5) the appearance and distribution of the white rot disease of certain species of *Allium* (*Sclerotium cepivorum* Berk.) was discussed. Its widespread occurrence in western Europe generally is known, while its recent development in the United States has become a matter of immediate concern to the onion-growing industry. From a general survey of its distribution in Europe one is led to surmise that the disease is most destructive in the cooler portions of the growing season and that its progress is checked rather than enhanced by the warmer mid-summer weather. Likewise, it is of interest to note that the first establishment of the causal organism as a pathogen of economic significance in America was in eastern Virginia, where it attacked the winter-grown crop of multiplier onion. A third report of the disease in America was made in May, 1925, from Louisville, Kentucky (3).

Because of the imminent danger of the establishment of this disease in other onion-growing sections of the country, the writer became interested in undertaking a laboratory study of the influence of certain soil environmental factors upon the disease. At first, attention was given to soil temperature and soil moisture, and this paper deals primarily with the results of those studies.

A number of strains of *Sclerotium cepivorum* were collected. Most of them were isolated by the writer from collections made in Europe or from materials collected by others in America. Their several sources and the number by which they will be designated in this paper are as follows: No. 2, isolated from garlic collected in May, 1922, at Bañolas, Catalonia, Spain; No. 3, isolated from onion collected in June, 1922, in Brittany; No. 4, isolated from onion collected in June, 1922, on the island of Teneriffe, Canaries; No. 5, isolated from onion collected in September, 1922, on the island of Overflakkee, Holland; No. 6, isolated by H. H. Whetzel from multiplier onion or garlic collected at La Grange, Oregon; No. 7, forwarded by Professor Whetzel, who obtained the culture from Dr. Pethybridge in Ireland; No. 8, culture from Professor Whetzel, who isolated it from infected onions sent him by A. D. Cotton, Kew, England; No. 9, isolated from multiplier onion collected by Herbert Spencer, near Norfolk, Va.; No. 10, isolated from onion forwarded by W. D. Valleau from collections made

near Louisville, Kentucky. These strains behave very much alike in their growth upon potato dextrose agar. The greatest aberration in macroscopic appearance was noted in strain No. 9 which produced an unusually profuse aerial white mycelium which tended to obscure the sclerotia located upon the surface of the substrate. All of these strains, with the exception of No. 3, proved to be pathogenic to onion when introduced into soil in which the host plant was later grown.

TEMPERATURE STUDIES

The relation of temperature to growth of the cultures upon potato dextrose agar was studied. The cardinal points for growth were found to be very nearly the same for all forms. Very slight growth occurred at 4–5° C., and it was usually not macrsocopically visible within a fortnight. The most prompt development occurred between 20° and 24° C., and, on the basis of rate and amount of growth, the optimum was somewhere between these points. Although the rate diminished as the temperature was lowered, there was quite as much growth at 15° C. in a somewhat longer period. At 27° C. growth was distinctly retarded; strain No. 6 was more tolerant to this temperature than any of the other strains. At 28.5–29.5° C. only very meager growth occurred in most of the strains during a two-weeks period; strain No. 4 did not grow at all. At 32–34° C. none of the strains grew. The maximum temperature for growth on potato dextrose agar therefore, was, in the neighborhood of 29° C., the minimum somewhere below 5° C. while the optimum was around 20° to 24° C. With the exception of a slightly greater tolerance to higher temperatures by strain No. 6, the various cultures reacted quite similarly to the various temperatures. The results of a single experiment are illustrated in Plate XXXV, A.

The obviously important question is that of the relation of temperature to infection and to the progress of the disease. Since the subterranean parts of the host are the ones first subject to invasion, the soil temperature relation is one of primary interest. Because of the similarity of the strains in their temperature reactions on culture media, most of the further work was conducted with a single strain, No. 5 from Holland having been selected. The sclerotia from pure cultures were mixed with virgin soil and several crops of onions grown until it was certain that the soil was thoroughly infested. Pot cultures were then started in galvanized iron cylindrical pots which were held at various desired temperatures in Wisconsin temperature tanks. Onion seedlings, grown to the third or fourth leaf stage, or onion sets were then planted in the soil. In certain cases bulbs or seedlings of other species of *Allium* as well were used in the experiment. The soil moisture in these series was adjusted so as to support good growth of the host and was kept reasonably constant by frequent weighing of the pots and replacement of lost water.

TABLE 1.—*Development of white rot in Crystal Wax Bermuda onion seedlings held at various soil temperatures*

Soil temperature in degrees C.	Inoculated soil					Uninoculated soil		
	No. plants used	After 23 days		After 40 days		No. plants used	After 40 days	
		No. healthy	No. diseased	No. healthy	No. diseased		No. healthy	No. diseased
14	10	0	10	0	10	5	5	0
18	10	0	10	0	10	5	5	0
22	10	7	3	5	5	5	5	0
24	10	8	2	5	5	5	5	0
26	10	10	0	10	0	5	5	0
30	10	10	0	10	0	5	5	0

Crystal Wax Bermuda seedlings in which four true leaves had emerged, were used in the first experiment. These were transplanted to pots of inoculated soil, and a certain number were transferred to pots of uninoculated soil. They were then placed in tanks which were kept at various constant temperatures. The air temperature of the greenhouse varied from 15° to 20° C. Although the temperatures of the water bath in the tanks varied less than one degree above or below the desired point, the soil at the surface of the pots, not being protected from the influence of the air temperature, varied somewhat more than this. The temperature of the surface inch of soil in the pots held at 14° C. therefore tended to run somewhat higher than the remainder of the pot, while in the case of pots kept at temperatures above that of the air the surface soil tended to run somewhat lower than the average for a given pot. Since the infection of the host takes place chiefly in the upper part of the pots, this error is to be considered in interpreting the final data. The disease appeared in the 14°, 18°, and 22° C. tanks on or before the thirteenth day. The results at the end of 23 days and 40 days are given in table 1.

The disease developed most rapidly at 14° and 18° C. At the end of the 40-day period all plants in these tanks were dead. At the two intermediate temperatures, 22° and 24° C., 50 per cent or less of the plants were affected, and not all of the diseased plants had succumbed at the end of 40 days. At 26° and 30° C. no infection occurred.

A second experiment of similar nature was conducted in which seedlings of three varieties of onion were used. Since the tops of the pots were not protected, the discrepancy in temperature between the upper layer of soil and the remainder of the pot still obtained. The limits of variation were therefore recorded. The results at the end of 80 days are recorded in table 2.

Infection proceeded equally well in each of the three varieties, but the influence of temperature was again evident. The progress was greatest at the lower temperatures, as in the previous experiment. At 23° C. and above there was again a decided limitation in infection. Representative pots of Red Globe variety in inoculated and uninoculated soil at different soil temperatures are illustrated in Plate XXXIV.

In order to eliminate the variation in the soil temperature at the top of the pot which prevailed in the experiments just described. two more series were conducted in which this factor was largely controlled. This was done by covering the soil with a layer of mineral wool as soon as the plantings were made, a procedure which served to exclude the influence of the air temperature upon the soil, and provided a more nearly uniform soil temperature throughout the pots. The soil was thoroughly mixed before potting so as to insure uniform distribution of moisture. As in previous

TABLE 2.—*Development of white rot in seedlings of Red Globe, White Globe and Crystal Wax onions at various soil temperatures; duration of experiment, 80 days; air temperature, 20–25° C.*

Soil temperature in degrees C.	Temperature range in degrees C. in upper layers of soil	Inoculated soil						Uninoculated soil			
		Red Globe		White Globe		Crystal Wax		Red Globe		Crystal Wax	
		No. of plants	No. diseased	No. of plants	No. diseased	No. of plants	No. diseased	No. of plants	No. diseased	No. of plants	No. diseased
10	10–11	14	14	18	18	18	18	9	0	9	0
14	14–15	18	17	18	15	18	18	9	0	9	0
18	18	18	18	18	18	18	18	9	0	9	0
22	20–22	18	10	18	15	18	16	9	0	9	0
26	23–26	18	1	18	1	18	1	9	0	9	0
30	25–30	17	0	17	17	17	0	8	0	7	0

experiments, the moisture was kept approximately constant during the course of each experiment by frequent weighing of each pot and replacement of lost water. In the third experiment Crystal Wax Bermuda seedlings were used, and the air temperature was somewhat higher than in the other experiments reported. In the fourth experiment the observations were extended to certain other susceptible species of *Allium*. Bulbs of garlic (*Allium sativum*), white multiplier onions (*Allium cepa* var. *multiplicans*), White Portugal onion (*Allium cepa*), and seedlings of White Welch onion (*Allium fistulosum*) were used. The final data collected are recorded in tables 3 and 4.

With the maintenance of uniform temperature throughout the pots, more critical judgment of the aggressiveness of attack at various points in the temperature range was possible. In the third experiment the disease seemed to develop with nearly equal severity from 10° to 20° C. (see table 3). If there was any variation within this range it would appear that the progress and destructiveness of the organism was somewhat greater at 16° C. than at temperatures above or below that point. With the more accurate control of temperature the exact point at which infection is inhibited becomes more apparent. Comparing the evidence at 20° and 22° C. it is to be seen that at the former temperature early infection occurred and all of the plants succumbed, while at the slightly higher temperature the appearance of the disease symptoms was not only delayed but the infection was confined to only one of ten plants. Constant temperatures of 24° C. and above were sufficient to prevent invasion by the parasite.

In the fourth experiment (table 4) the reactions of garlic, Welch onion, multiplier onion, and common onion were studied. The largest percentages of infection and the greatest advance of the disease were found at 14° and 18° C., showing that variation in host plant had no apparent bearing on the temperature relation. The 8–10° C. series was lower than any maintained in previous experiments, and inhibition of infection at these temperatures was evident with all four host plants. The absence of disease above 22° C. was noteworthy in each species of *Allium* tested. The reduced percentage of infection in the case of the multiplier onion is significant and suggests the possibility of variation of susceptibilty among known host species or varieties. Cotton and Owen (1) have already recorded some observations along this line.

The temperature reaction of a parasitic fungus as indicated by its vegetative growth on a favorable culture medium is not always a safe criterion for predicting the relation of temperature to its pathogenic properties. In this case the discrepancy is noteworthy. Although the greatest amount of vegetative growth on potato dextrose agar in a given period was above 20° C., infection was greatly retarded beyond this point. Nearly as good

TABLE 3.—*Development of white rot in Crystal Wax Bermuda onion seedlings at various soil temperatures; air temperature 15–20° C.*

Soil temperature in degrees C.	Inoculated soil					Uninoculated soil		
	No. of plants used	After 26 days		After 40 days		No. of plants used	After 40 days	
		No. healthy	No. diseased	No. healthy	No. diseased		No. healthy	No. diseased
10	10	2	8	1	9	10	10	0
13	10	3	7	0	10	9	9	0
16	10	0	10	0	10	10	10	0
18	10	4	6	2	8	10	10	0
20	10	2	8	0	10	9	9	0
22	10	10	0	9	1	9	9	0
24	9	9	0	7[a]	0	10	10	0
26	9	9	0	9	0	8	8	0
28	9	9	0	9	0	8	8	0
30	9	9	0	9	0	9	9	0

[a] Two died from other causes.

TABLE 4.—*Development of white rot in garlic, white multiplier, White Portugal, and White Welch onions at various soil temperatures. Duration of experiment 73 days*

Soil temperature in degrees C.	Garlic		White Multiplier		White Welch		White Portugal	
	Total no. plants	No. diseased	Total no. plants	No. diseased	Total no. plants	No. diseased	Total no. plants	No. diseased
8–10	8	0	9	2	10	3	10	7
14	8	7	10	2	10	7	10	10
18	8	6	9	4	10	9	10	10
20–22	10	1	8	1	10	2	10	3
26	9	0	5	0	10	0	10	0
28–30	10	0	10	0	10	0	10	0

growth was secured between 15° and 20° C. after a somewhat longer time had elapsed. The fungus is a very active pathogen, however, from 10° to 20° C. There appears to be little connection between rate of growth on potato dextrose agar and pathogenicity.

In this connection it may be well to consider the influence of soil temperature upon the development of the host. Some earlier data were given on the growth of young seedlings of Red Globe and Yellow Bermuda onions during the first 30 days after sowing (6). In the Red Globe (a northern variety) the best development of roots was found at soil temperatures below 20° C., while the best development of tops was at 20° C. and above. In the Yellow Bermuda (a southern variety) the best root growth was at 20° C. and the best top growth at 25° C. In the first soil temperature experiment reported in this paper the control plants in uninoculated soil were carefully washed out of the soil after 52 days and the dry weights of the roots and tops determined. At 22° C. and above the plants had formed distinct bulbs, while at 14° and 18° C. the leaf bases were just starting to thicken. The bulb and the remainder of the top were dried separately in each case. At the same time a series of plants from White Portugal sets was run for a slightly shorter period in uninoculated soil. The dry weights of these were likewise procured. In table 5 the results of the two lots are summarized. The best root development, as in the young seedlings, was at 22° C. or below while in general the best bulb development was at

TABLE 5.—*Dry weights of roots, bulbs, and leaves of White Portugal and Crystal Wax Bermuda onions held at various constant soil temperatures, but exposed to the same air temperature (15–20° C.)*

Variety, condition, and dates	Soil temperature °C.	Av. wt. of roots (grams)	Av. wt. of bulbs (grams)	Av. wt. of leaves (grams)	Av. wt. of bulbs and leaves (grams)	Av. wt. of total plants (grams)
White Portugal plants started from sets. April 16 to May 24.	10	.307	.713	1.196	1.909	2.216
	14	.250	.947	1.257	2.204	2.454
	18	.198	1.365	1.692	3.057	3.255
	22	.283	1.649	1.678	3.327	3.610
	26	.099	1.527	1.054	2.581	2.680
	30	.064	1.341	1.227	2.568	2.632
Crystal Wax Bermuda seedlings in 4th leaf and about 6 inches high at time of transplanting to pots. April 14 to June 5.	14	.118	.145	.508	.652	.770
	18	.135	.286	.525	.810	.945
	22	.100	.615	.546	1.160	1.260
	24	.066	.843	.288	1.131	1.197
	26	.040	.637	.117	.754	.794
	30	.018	.734	.080	.814	.832

22° C. and above. As a whole the most rapid growth occurred in both varieties at 22° C. The critical point, then, at which infection is abruptly inhibited is not that at which there is an evident check in the growth of the fungus in pure culture, but that at which the host is growing most vigorously. One may be led to question, therefore, whether or not the influence of temperature upon the host metabolism may not have an indirect bearing upon the relation between host and parasite.

SOIL MOISTURE RELATIONS

In the foregoing experiments the influence of temperature was the primary consideration. In each case the soil moisture was approximately the same throughout the experiment, but no attention was given to the influence of different amounts of soil moisture upon infection. The next experiment was planned in order to study the latter question. The same soil was used as in previous experiments, and division into four lots was made. The water-holding capacity of the soil was found to be 60 per cent of the dry weight. The four lots of soil were then adjusted respectively to moisture contents approximating 80, 60, 40, and 20 per cent of the water-holding capacity. The soil was placed in galvanized iron cylindrical pots 5 inches in diameter and with a depth of soil of about 5 inches. Three pots were made up for each soil moisture. The pots were then planted with White Portugal sets and placed in a greenhouse at a temperature of 15-20° C., which was within the range at which best development of the disease had previously been found to occur. The pots were weighed daily and sufficient water added to bring each up to the original weight. This method of maintaining soil moisture is of course subject to some error, and variation within the pot is to be expected. It is practically impossible to compensate accurately for the increase in weight due to the growth of the plants, so no attempt was made to correct this error. As a result, when the soil moistures were determined at the end of the experiment, it was found that the moisture content had been reduced somewhat in the wetter soil where the greatest amount of growth occurred.

The experiment was made over a period of 48 days. The soil moistures at the beginning and end of the period, together with the amount of disease, are given in table 6.

The lowest moisture content, 13 per cent, was not sufficient to promote anything but very meager growth and was entirely too low for normal development of the plant. Infection also was apparently retarded by the lack of sufficient moisture. The top growth of the onions increased with the soil moisture, and the rankest leaf development occurred in the highest soil moisture. The plants grew well at 23 per cent moisture content, but infection occurred here first and the disease progressed most rapidly. At

TABLE 6.—*Relation of soil moisture to development of white rot in White Portugal onions; duration of experiment, 48 days; water-holding capacity of the soil, 60 per cent*

Lot No.	Moisture content of soil in percentage		Condition of plants at end of experiment		
	Beginning of experiment	End of experiment	Total no. plants used	No. healthy	No. diseased
1	13	12	24	12	12
2	23	26	24	0	24
3	37	30	24	19	5
4	48	37	22	20	1

the conclusion of the experiment all of the plants at this temperature were affected and most of them were dead.

The behavior of the plants at the higher soil moistures is also noteworthy. In lot 3, where the soil moisture was kept within 50 to 60 per cent of the water-holding capacity, 20 per cent of the plants were diseased as compared with 100 per cent in the soil with a moisture content of 23 per cent. In lot 4, where soil moisture was raised to a still higher point, the amount of infection was still less. Representative pots from each lot are illustrated in Plate XXXV, B.

The healthy plants were just beginning to bulb in lots 3 and 4 at 48 days. The moisture of the soil was raised again to 37 and 48 per cent respectively, the pots being kept at constant weights as before, and most of the plants were allowed to continue growth for another 53 days. By that time bulbs had formed averaging about one inch in diameter. One more plant in lot 3 had succumbed to white rot during that period, while in lot 4 one plant was infected at the neck of the bulb. All the remaining plants were apparently sound.

It is thus evident that the amount of moisture in the soil may greatly affect the severity of the disease even at the optimum temperature. It is interesting to note that within the temperature range favorable to growth of the plant, the fungus becomes less destructive as the rapidity of host growth increases. There remains for further investigation the question of whether this shift in soil environment constitutes a direct retardation of the parasite or a stimulation of greater resistance as a result of metabolic changes in the host, or both.

DISCUSSION

The data which have been presented establish the fact that soil temperature has a marked influence upon the development of white rot. The latter

may be properly classed as a low temperature disease, and its greatest destruction may be looked for when the average soil temperature is 20° C., or below; while at temperatures above 22° C. the host may be expected to escape infection. In addition to temperature, the soil moisture is equally important. The disease may be expected to occur at as low soil moisture as will support growth of the plant, while in soil where the moisture is above 50 per cent of the water-holding capacity a gradual reduction in infection will occur as the moisture is raised even though the temperature is at the optimum for the pathogenicity of the organism. Just how the influence of these soil environmental factors is exerted upon the parasitic relation remains an unanswered question. It is pointed out that the optimum temperature for growth of the fungus on potato dextrose agar lies in a range where little infection occurs. Is there an optimum temperature for vegetative growth on potato dextrose agar and another for the expression of its pathogenic properties in the soil habitat? Or is the metabolism of the host at the higher temperatures the limiting factor? In the same way, are we to attribute the reduction of the disease in the wetter soil to inhibition of the parasite or to stimulation of the host?

On the questions of general distribution and destructiveness of the disease, the facts have an important practical bearing. The prevailing soil moisture is likely to be extremely variable throughout the season in a given locality, and it is quite probable that such variations have their effects on the development of the disease. With regard to soil temperature it is easier to predict that certain localities where onions are grown offer more favorable conditions than others. For instance, in the northern onion-growing sections of the United States the crop is subjected to ideal temperature conditions for white rot during the early part of the growing season while following midseason the soil temperature is likely to be quite unfavorable. Such records as have been accumulated for southeastern Wisconsin (4, 5), where onion sowing is done, as a rule, in April, show that during May and part of June the daily mean temperature of the upper 2 inches of soil is usually below 20° C., while for the rest of the onion growing season it is usually above 20° C. In sections in the tier of states south of Wisconsin the period of favorable temperature is probably somewhat shorter. When one considers still more southerly sections, however, where the seed is commonly sown or sets planted in the autumn, it is quite likely that a longer period of favorable soil temperature prevails. Moreover in this region white rot has already become an established disease.

SUMMARY

The growth of the white rot organism (*Sclerotium cepivorum* Berk.) on potato dextrose agar occurs from somewhat below 5° C. to about 29° C. The

most rapid development takes place between 20° and 24° C. while it is nearly as great at 15° to 20° C. after a somewhat longer period of time.

Infection occurs readily and the disease progresses most rapidly at soil temperatures between 10° and 20° C. At 20° and 22° C. there is a marked reduction in infection, and at constant temperatures of 24° C., or above, the disease does not appear. This temperature reaction holds for three varieties of common onion; multiplier onion, Welch onion, and garlic.

In the soil used, the greatest development of the disease occurred when the soil moisture was kept at about 40 per cent of the water-holding capacity. At 20 per cent of the water-holding capacity the growth of the host was very slow and the amount of disease was reduced. At 60 per cent and 80 per cent of water-holding capacity the growth of the onion plant was accelerated but infection was greatly reduced.

It is pointed out that, under natural conditions, variations in soil moisture may have a direct effect upon the occurrence of the disease. In the northern onion-growing sections of the United States the most favorable soil temperatures occur during the first half of the growing season while during the last half the daily mean soil temperature for the upper layers of soil is, for the most part, above the point most favorable for disease development (10°–20° C.). In the more southerly sections, where winter crops of onion and related plants are commonly grown, the favorable temperatures probably extend over a long portion of the growing season.

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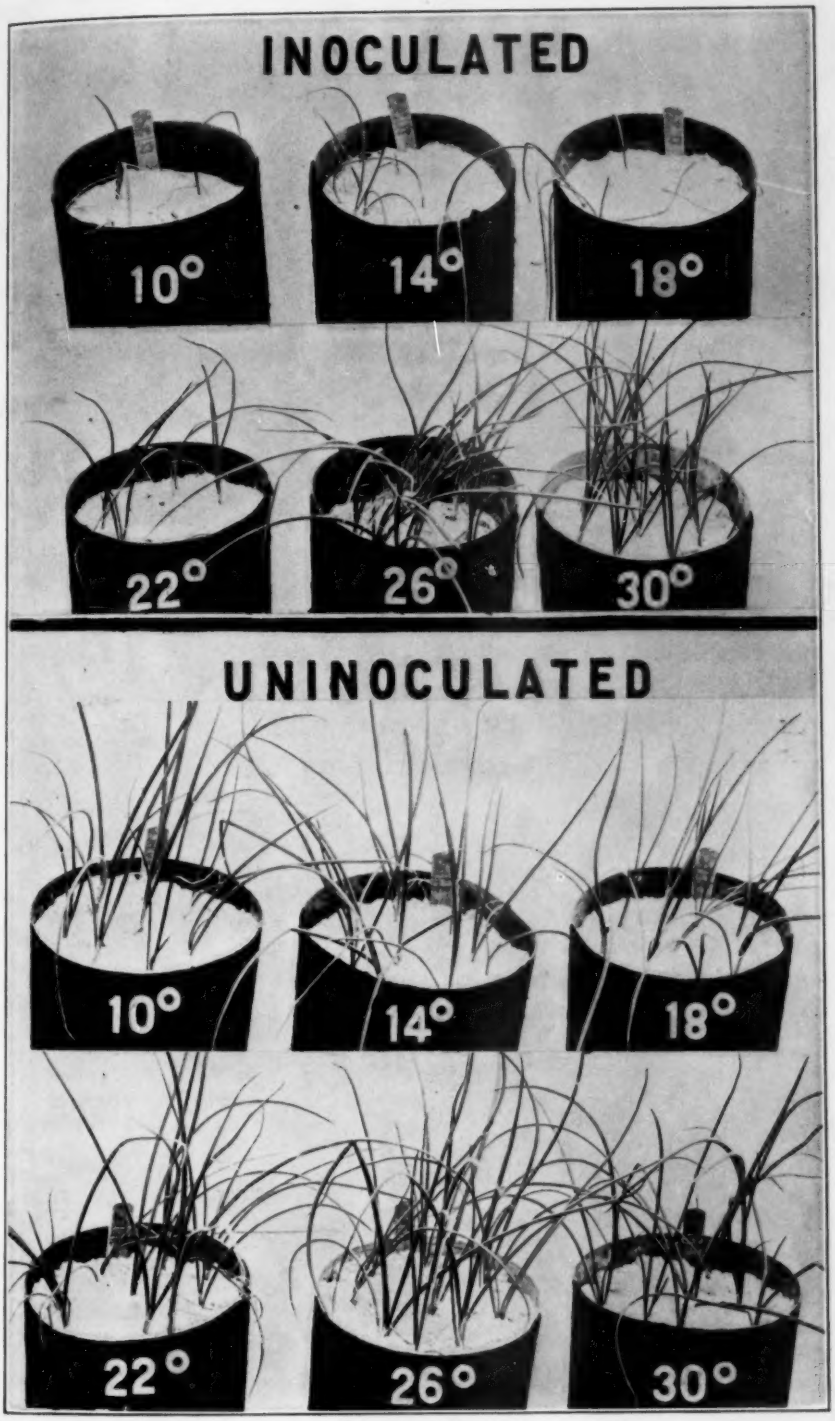
EXPLANATION OF PLATES

Plate XXXIV. Red Globe onion seedlings 40 days after being transplanted to soil inoculated with *Sclerotium cepivorum* and to uninoculated soil. The soil was held at the temperatures indicated, and the air temperature varied from 15° to 20° C. Since the tops of the pots were not covered in this experiment, there was more variation in the upper layers of soil than in later experiments. At this stage the fungus had destroyed most of the plants at 10°, 14°, and 18° C. soil temperature, while in the 22° C. tank, where the upper soil varied between 20° and 22° C., the progress of the disease was not so great. At 26° and 30° C. the plants were free from infection. In subsequent experiments in which the soil was covered with mineral wool and more uniform temperature maintained throughout the pot no disease occurred above 22° C.

Plate XXXV. A. Growth of *Sclerotium cepivorum* on potato dextrose agar plates after a period of 16 days at the various temperatures indicated.

B. Onions grown from sets planted in soil inoculated with *Sclerotium cepivorum* and held at various degrees of soil moisture for 48 days. The water-holding capacity of the soil was 60 per cent of the dry weight. The moisture content of the soil in the pots was as follows: (1) 12–13 per cent. (2) 23–26 per cent. (3)30–37 per cent. (4) 37–48 per cent.

In lot 1, where there was insufficient moisture to support good growth, 50 per cent of the plants were diseased; in lot 2, 100 per cent were diseased; in lot 3, 20 per cent; in lot 4, 5 per cent.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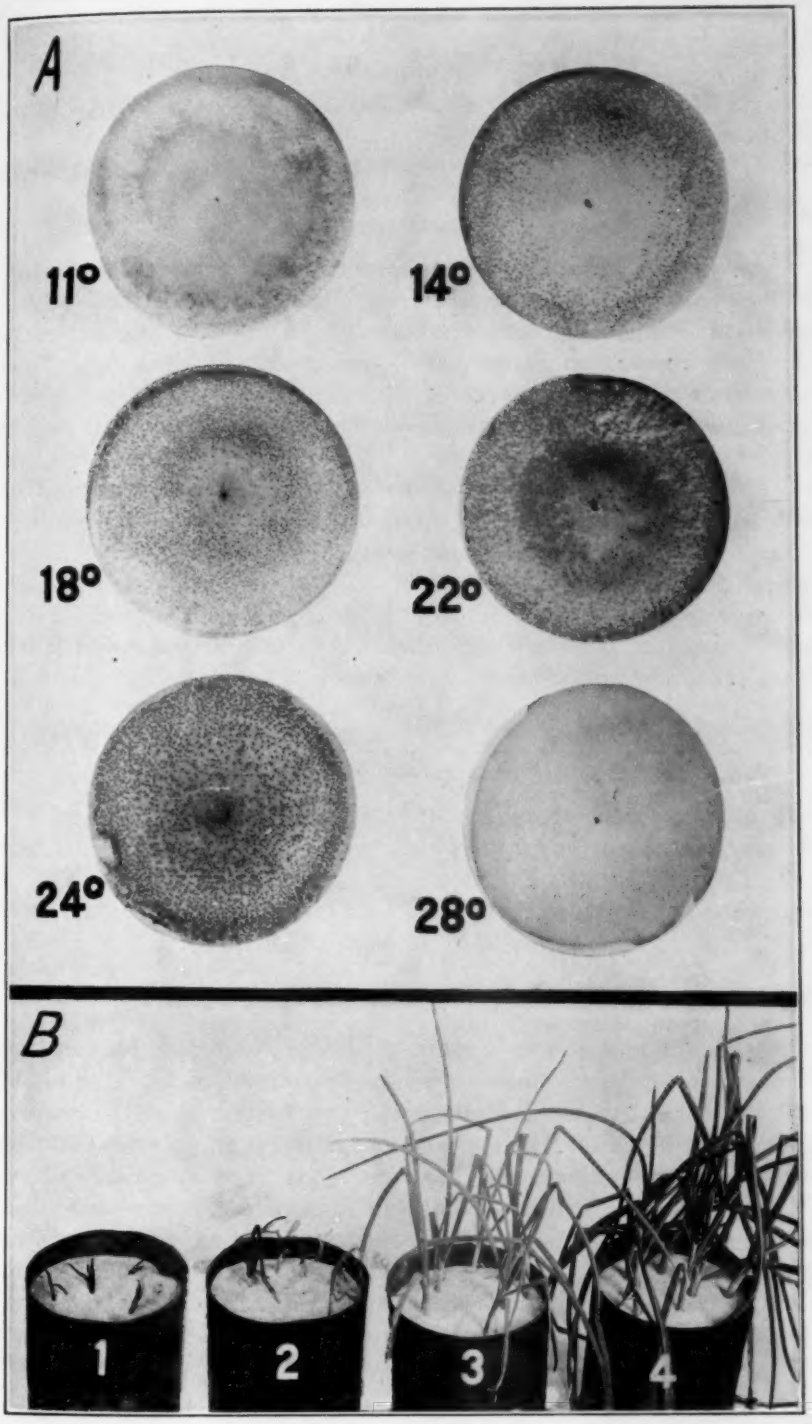
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SPRAIN OR INTERNAL BROWN SPOT OF POTATOES

D. ATANASOFF

COMMON NAMES

In Holland, sprain or internal brown spot of potatoes is generally known under the name "Kringerigheid" (ringiness). In the German speaking countries the disease is known under a large variety of names. Of these "Eisen" (50) and "Buntfleckigkeit" are perhaps most commonly used. But the disease is also known under the following names: Buntwerden der Kartoffeln, Pfropfenkrankheit, Braunfleckigkeit (59), Rostfleckigkeit, Ringbräune, etc.

In England the same disease is commonly called "sprain" (37). In 1910 Horne (19) distinguished two forms of this disease: "internal disease" and "sprain" or "streak" disease; and, in 1914 (20), "blotch" and "streak." Paine in 1918 (33) and 1923 (34) used the name "internal rust spot" for the disease. In the United States it is commonly called "internal brown spot" (32), occasionally also "internal browning." In South Africa (12) the name "internal brown fleck" is applied to the same disease.

GEOGRAPHIC DISTRIBUTION

The internal brown spot disease of the potato is quite generally distributed wherever potatoes are grown. It has been reported from various places in Holland, Germany, Denmark, Sweden, Norway, Austria, Czechoslovakia, Great Britain, the Dutch East Indies, South Africa, and numerous localities in the United States of America and Canada.

ECONOMIC IMPORTANCE

As a rule internal brown spot is of minor economic importance. Only in certain soils and in certain years does it assume considerable economic importance. In such cases it may practically ruin the crop as far as the consumptive value of the tubers is concerned, though their value as animal food or for starch production is not materially decreased even when severely affected. Cases where the disease decreased the yield of marketable tubers as much as 50 per cent and even more often have been reported (47). During the summer of 1924 the writer observed that more than 50 per cent of the tubers from a potato field in Bennekom, planted with the very susceptible varieties Schotsche Muis and Eigenheimer, were severely affected

and were unfit for human consumption (Fig. 1). Paravicini (35) reports that in some regions in the Dutch East Indies from 80 to 100 per cent of the tubers may become affected.

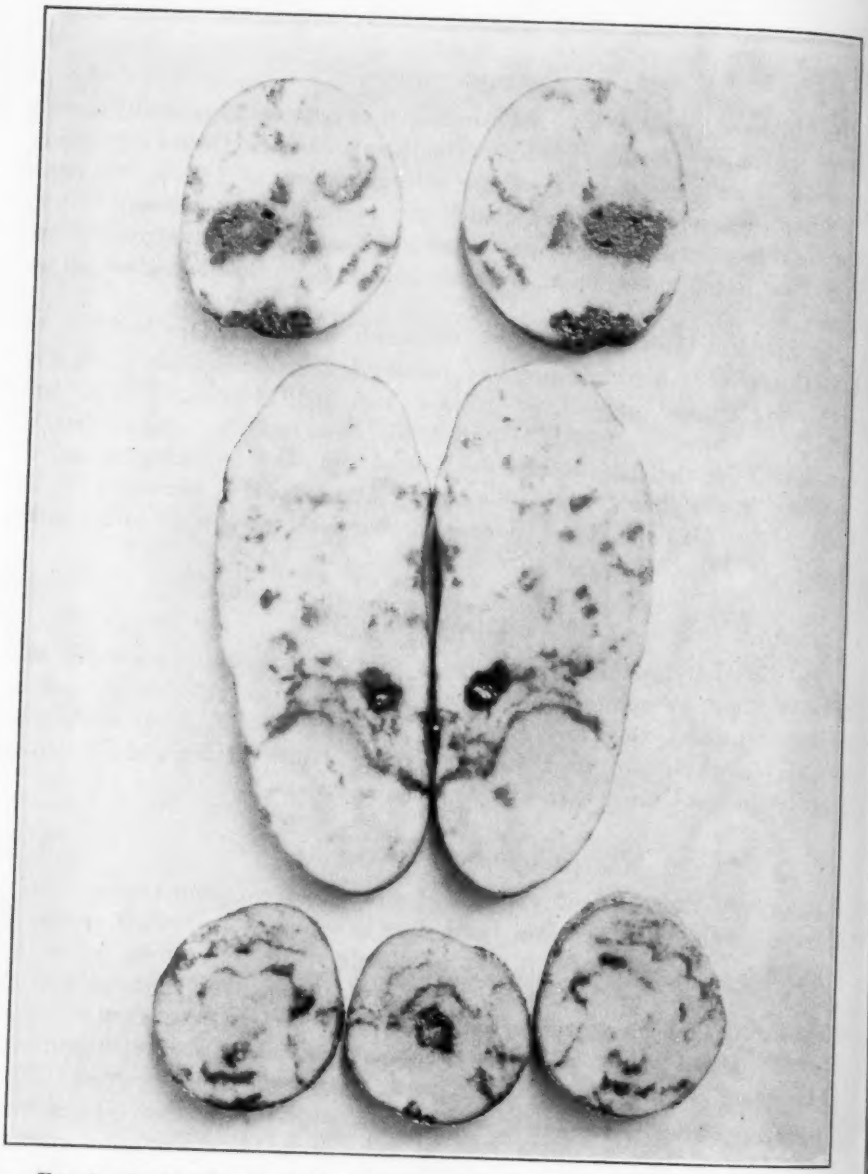

FIG. 1. Cross and longitudinal sections of Schotsche Muis (Midlothian Early) tubers, showing internal brown spot symptoms.

DESCRIPTION OF THE DISEASE

In recent years it has been shown that brown spotting of the flesh of potato tubers is not only characteristic of the internal brown spot disease but may be brought about also by numerous other diseases and conditions, *e.g.*, frost necrosis (24), net necrosis (32), stipple-streak (Atanasoff), etc. Much of what has been considered up to now as internal brown spot has been net necrosis. Therefore, an exact and detailed description of the disease in question seems desirable.

External Symptoms. The disease appears in the tubers long before they are fully grown or lifted, but it is never, or only seldom, seen in the very young tubers.

In most cases the affected tubers show no external symptoms of the disease whatever. Such is the case with tubers which have been attacked by the disease in a more advanced stage of their development or shortly before ceasing to grow, also of tubers on the market and in storage. Such tubers are dirty, somewhat discolored, and often more or less bruised and injured; thus the external symptoms of the disease are obscured. However, when the tubers are dug carefully and rinsed in water, there will be seen on some of them, and especially on those attacked at an early stage of their development, some very distinct changes. This is especially true of the more susceptible varieties and of those with light-colored skin. Tubers that are attacked by the disease while still rapidly growing have an irregular outline (Fig. 2) and show more or less pronounced deformations and depressions. These depressions may be so deep as to give the tuber a very irregular or semicircular shape. The skin over the depressions is much thicker and rough, often cracking in all directions (Plate XXXVI, A), the cracks giving the tuber a scabby appearance. If the skin over the depressions is not too thick and brown colored, there may be seen, as first reported by Pethybridge (37), dark or chocolate brown blotches underneath in the flesh of the tuber (Plate XXXVI, B). In some of the more susceptible early varieties, as Schotsche Muis for instance, these blotches may be very large and extend deep into the tuber. In such cases secondary organisms develop in the dead tissues and give rise to a large brown or black lesion on the tuber (Fig. 1).

Tubers attacked by the disease shortly before or after reaching their full development are quite normal in shape and size. Their skin, however, shows here and there slight irregularities and rough spots, under which are visible, in the case of newly dug tubers, brown blotches, spots, streaks, or ring-like discolorations of the flesh, which seem to be arranged around a central spot, lying usually under a lenticel. These central spots, as well as the centers of the depressions, apparently represent the original point of infection. When the skin is removed carefully, the discolorations in the flesh of the tuber become even more distinct.

Internal macroscopic symptoms. Upon cutting a tuber longitudinally or in cross section there will be seen dark rusty brown blotches or streaks irregularly spread over the cut surface and having no connection with the vascular ring, stem-end or eyes of the tuber nor apparently with the periderm of the tuber, as observed by numerous authors (Fig. 1). In reality this is not so. When the tubers are cut perpendicularly through the externally visible brown areas, or through the depressions (Fig. 2), which apparently represent the original point of infection, it will be seen that the rusty brown discolorations of the flesh radiate from the center of the depressions or externally visible brown blotches underneath the skin. In tubers infected simultaneously at several points, where the rusty brown blotches, streaks, or rings are numerous and all over the tuber, the above condition is much less pronounced. This is also true of tubers in an advanced stage of the disease (Fig. 1).

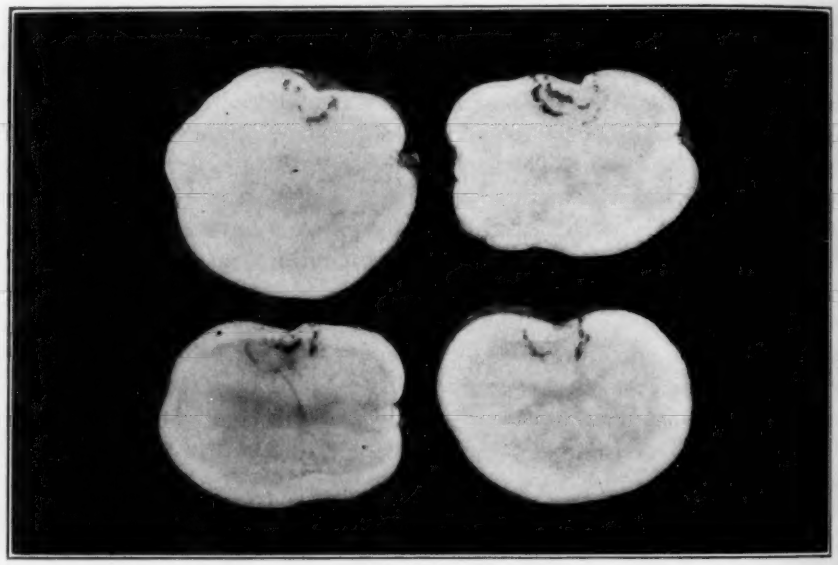

FIG. 2. Longitudinal sections of Eigenheimer tubers showing internal brown spot symptoms. Tubers cut through the depressions, which represent the point of infection. Note the concentric rings and spots around the points of infection.

The form of the brown discolorations may be of two kinds, according to the variety and presumably also to other not fully understood factors. In some varieties they consist mostly or entirely of irregular, rusty brown blotches of varying size and shape. This form of the disease may be mistaken, in superficial examination, for net necrosis. In other varieties, even when growing under exactly the same conditions, the discolorations have

usually or exclusively the shape of streaks, arcs, or rings, always radiating from a certain center (Plate XXXVI). The latter usually lie, in the early stages of the disease, at the surface of the tuber; and it is not unusual to see several brown arcs or more or less complete rings formed concentrically. The rusty brown areas lie more or less sharply outlined in the normal tuber tissues.

The number and extent of the rusty spots and of the browning usually increase considerably during the first months subsequent to the lifting of the potatoes, and remain so during the rest of the life of the tuber. The increasing of the browning is more apparent in the early-lifted tubers than in tubers which have been dug late and in fully ripe condition.

Infected tubers of most varieties, when properly stored, usually will keep as well as healthy tubers. Severely affected tubers, primarily of the more susceptible varieties as Schotsche Muis (Fig. 3) and Eigenheimer, which show deep lesions at the time of lifting, undergo a kind of dry rot during the winter, whereby the whole interior of the tuber becomes brown and perforated by numerous cavities. The dead tissues and cavities are gradually invaded by fungi, mostly Fusaria, and the whole tuber finally becomes a hard mummy.

Internal microscopic symptoms. Hand sections through the brown patches show that they consist of groups of otherwise normal but rusty or reddish brown cells which in most cases are packed with starch grains. These cells are dead. The cell contents, except the starch grains, are shrunken, brown and indistinguishable, while the cell walls seem to be considerably thickened and suberised. No foreign organisms such as fungi, bacteria, etc., are visible in the dead tissues. The brown patches may be of considerable size or may consist of only a few cells. In most cases they are surrounded by a distinct cork layer, consisting of three to six or even more layers of thin cork cells. This is especially true some time after the lifting of the plants. The cork layers, as well as the neighboring cells of the surrounding healthy tissues, are starch-free and translucent, so that a light ring becomes apparent around each blotch when the sections are examined in transmitted light.

ETIOLOGY OF THE DISEASE

The absence of visible pathogenes in the rusty brown tissues of the affected tubers led the students of this disease from the very beginning to consider it to be physiological in nature, presumably caused by disturbed chemical equilibrium in the plant, or by soil or climatic conditions. This is now the most current opinion regarding the nature of internal brown spot.

Former investigations. Numerous workers have searched for the pathogene of this disease, but Swellengrebel in Holland and Paine in England are the only two workers who claim to have succeeded.

Swellengrebel (52), in an extensive paper on this subject, was the first to attribute the cause of the disease to bacteria. By removing portions of the brown tuber tissues under aseptic conditions and placing them on sterile boiled potato plugs, Swellengrebel found that the affected tissues are not sterile, as claimed by former workers, but contain bacteria. In most cases he isolated the following four bacteria: *Bacillus megatherium* De Bary, *Bacillus vulgatus* Migula, *Bacillus mesentericus* L. et N., and *Bacterium punctatum* L. et N. Swellengrebel considers these bacteria not as specific pathogenes, but as harmless saprophytes present everywhere in the soil and on the potato tubers, under ordinary conditions harmless, but evidently

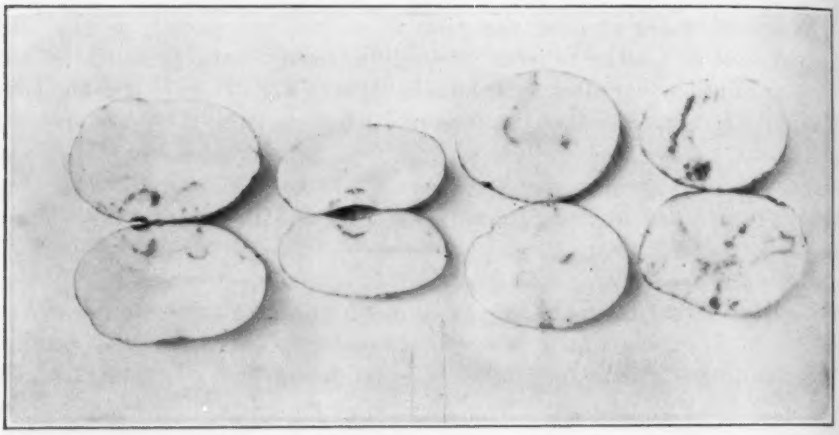

FIG. 3. Schotsche Muis (Midlothian Early) tubers cut longitudinally to show the symptoms of internal brown spot.

becoming parasitic under certain conditions. New tubers inoculated with pure cultures of the four bacteria produced, after several months, in some cases a rusty brown discoloration of the tissues around the wound in which the inoculum was deposited. The brown tissues showed the same microscopic picture as those of tubers naturally infected with internal brown spot. This browning of several cell layers around the point of inoculation, which did not appear in the control tubers, was considered by Swellengrebel as proof that each of the above four organisms independently is able to cause the disease.

Paine, in 1918 (33) and 1923 (34), reported that he had repeatedly isolated a bacterium from the affected tissues of diseased tubers. This organism, which he calls *Pseudomonas solaniolens*, is a small, oval, non-sporing rod, motile by a very long, single polar flagellum, and has a penetrating smell of earthy potato.

Paine infected tubers by inoculating with cultures of this organism inserted into a hole in the tuber, two or three centimeters deep and half a centimeter wide, made with a sterile carpenter's auger. The hole was plugged with a core of potato cut with a sterile cork-borer from another tuber of the same variety. The inoculated tuber was either planted in soil or kept in a waxed cardboard box. "After three or four weeks incubation of material inoculated in this way one finds the core reduced to a hard shrivelled corky mass, the hole usually widened towards the bottom and walls thickly suberised with a brown mass of cork having finger-like projections running out into the surrounding tissue; the suberised walls of the cavity are usually about two to three millimeters thick." The suberization, Paine finds, is exactly like that of the naturally affected tubers. "In two or three experiments threads of cork have been traced running into the tuber for more than a centimeter, and these exactly resemble those of net necrosis. We thus have both types 'A' and 'B' arising from the same inoculation." Paine (34) distinguishes two types of the disease: type 'A' represents the internal brown spot proper, and type 'B' the disease known in the United States and England as "net necrosis."

EXPERIMENTAL METHODS OF ISOLATION

The writer repeated Swellengrebel's and Paine's work. In numerous isolation experiments he found that occasionally the affected tissues of the tubers contain bacteria. So far, however, none of the organisms isolated from diseased tubers have produced the symptoms of the disease observed in nature, although most of them have produced a local browning and suberization of the tuber tissues around the point of infection.

The freshly dug, but externally uninjured, diseased tubers[1] are thoroughly washed in water with soap and brush, then disinfected in 2 per cent $HgCl_2$ for 3 to 4 hours, and subsequently held in a flame and gently rotated so as to burn off the skin of the tuber on all sides. The tuber thus treated, so far held with forceps, is placed in the left hand, which previously has been thoroughly washed and disinfected. The tuber is held at one end and only with the tips of the fingers. The cortex of the tuber is then removed with a flame-sterilized, all-steel scalpel, which has been cooled by dipping in boiling water. From the peeled end of the tuber are cut square blocks, large enough to fit into a culture tube. The potato plugs are then placed in culture tubes containing about 10 c.c. of sterile water and held ready by a second person. A large number of such tubes are prepared each time. The tubes to which large portions of the affected tubers have thus been

[1] New tubers were used throughout the isolation work on account of the possibility that the pathogene might die out in the older tubers. Besides, the probability is much greater that old tubers will subsequently become invaded by saprophytic organisms.

added are incubated for several weeks at 20° C. Owing to the osmosis which takes place under such conditions, a considerable portion of the protoplasm and soluble cell contents of the potato plug passes into the water and makes it a highly suitable culture medium for the growth of bacteria and other organisms. That not only the soluble cell contents but also the cell protoplasm diffuses into the water can easily be shown by warming up such tubes. This causes a coagulation of the protoplasm, and the liquid content of the tubes becomes milky.

If any organisms actually are present in the affected tuber tissues, they most likely will appear in the liquid outside of the living potato plugs and become visible through clouding or other visible changes in the liquid. In fact a considerable number of the four hundred or more test tubes prepared in this way became clouded within two to three weeks owing to the development of bacteria in them. The percentage of clouded tubes varied from time to time from 10 to 30 per cent, while similar test tubes prepared from healthy tubers seldom showed any bacteria, although in both cases each time a small number of tubes became contaminated with *Penicillium*.

Infection experiments with bacterial cultures isolated in the above way from tubers affected with internal brown spot have so far given no conclusive results.

Pathologic changes in potato tubers as produced by Swellengrebel and Paine with the alleged pathogenes of this disease have been repeatedly produced by the writer, not only with cultures of different bacteria isolated from diseased tubers, but also with a large number of other bacteria isolated from soils where the disease never does occur, and from other sources. Often the characteristic rusty discoloration of the tissues around the point of infection was very pronounced and penetrated farther than in Paine's experiments. But in all cases the discoloration, though identical with that produced in naturally infected tubers, remained limited to the tissues surrounding the point of infection and never extended into the interior of the tuber as in the case of naturally infected tubers.

The inability to produce experimentally the symptoms of the disease as observed in nature can not be due to the fact that the environmental conditions in the experimental work, and the conditions of the tuber itself, differ from the conditions under which the disease develops in nature, as Paine (34) is inclined to believe. That this is so is shown by the fact that, when a piece of a naturally infected tuber is grafted to a newly dug or still fresh and healthy tuber, the disease will develop in the healthy tuber in a most typical form within two or three months, and brown blotches and typical rusty brown arcs and rings will appear far away from the graft in the parenchyma of the healthy tuber. This experiment has been done often by the writer, and, as long as the tubers used were new and fresh, each time with the same results.

It may be that some of the organisms isolated from diseased tubers are actually the cause of the disease. It is possible that they may rapidly lose their pathogenicity outside of the tuber or in artificial culture. In all cases the writer has used cultures isolated only from two to four weeks previously from affected tubers, but, as already stated, in all cases the results were negative. Moreover, it is reasonable to expect that organisms which are virulent enough to cause distinct and very typical browning of the tissues around the point of infection, occasionally to a depth of more than one centimeter, should be able, if identical with the cause of the disease, to penetrate farther and bring about the symptoms of the disease seen in naturally infected tubers.

DISCUSSION AND SUMMARY

The experimental evidence to date regarding the cause of the internal brown spot of the potato tuber is in no way convincing.

The following facts, however, show that it is not a physiological disease and throw some light on its nature:

1. Under ordinary conditions in Holland internal brown spot of potatoes occurs constantly in a more or less severe form on certain soils primarily rich in organic matter, or heavily manured, light, sandy loam, or sandy soils, and reclaimed muck lands. It has never been seen in Holland on heavy clay soils, even only a short distance from muck and sandy soils where the disease is very common.

2. The writer's observations during a period of five seasons have convinced him that lack of moisture can not be the cause of the disease, as is claimed by some. On the contrary, sufficient moisture seems to be a prerequisite for the development of this disease in severe form. The only year, out of five, during which the disease caused but little injury in a field where it severely attacked the tubers during the other four years, was the unusually dry summer of 1921. During this season the disease was practically absent. The early portion of the summer of 1925 was also very dry and warm. The early variety Schotsche Muis (Midlothian Early), which is very susceptible to internal brown spot, was perfectly free from it up to June 15, at which time the plants were almost ripe for lifting. Subsequent to June 15 continuous rains came and the air temperature fell. Ten days later numerous cases of internal brown spot occurred.

3. The disease is not transmitted with the potato seed.

4. The causal agent of the disease enters the tubers from without, bringing about distinct pathologic changes at the point of entrance to the tuber. These consist of distinct scabbing of the tuber periderm and slight or pronounced depressions in, and deformations of, the tubers. The subsequent browning of the tuber parenchyma radiates from the point of infection, usually visible on the outside of the tuber.

From the above it could be concluded that the cause of the disease is an organism or a chemical substance. The latter seems less probable, because it is not very likely that at certain points in the soil, especially in wet seasons, the concentration of a given substance can be so much higher than at other points around the tuber as to cause a local and not a general poisoning of the tuber surface.

5. The disease can easily be transmitted to fresh healthy tubers by grafting portions of diseased tubers on them. This also is in favor of the supposition that the disease is caused by a living organism and is not due to a local poisoning of the tuber. Were the latter the case, the poisonous substance which might have entered the first tuber and brought about the death of local areas would at last become so diluted or bound that it would not be able to pass to any extent into the grafted tuber, at least not deep into the interior of the healthy tubers, as was always the case in the writer's graft experiments. Even less probable seems the transmissibility of the effect of given unfavorable environmental conditions from the diseased tuber portions to the healthy tubers grafted with them.

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EXPLANATION OF PLATE XXXVI

A. Eigenheimer tubers showing scabbing at and around the point of infection with internal brown spot.

B. Same tubers with scabbed epidermis, removed to expose the points of infection, and the brown spots and rings radiating from them.

C. Same tubers cut through the point of infection. Note the arrangements of brown spots and rings around the points of infection.

A
B
C

THE MORPHOLOGY AND THE PATHOGENICITY OF SOME PHYTOPHTHORA MUTATIONS[1]

LEON H. LEONIAN

Although physiology forms the chief basis of bacteriological classification, mycologists still cling to the purely morphological methods. As a result of this conservative policy many groups of fungi are extremely confusing not only to the average investigator but even to the so-called specialist.

It is the purpose of this paper to demonstrate how easily one may, if strict morphological methods are followed, make new species almost at will, and how infinitely difficult it is to identify these species after they are once made.

The mutations of the rhubarb *Phytophthora* (formerly *P. parasitica* var. *rhei*, but more recently included[2] in *P. omnivora*) have been used for the purpose outlined in the foregoing paragraph. Plate XXXVII illustrates the colony characteristics of these five types. All of these organisms came from a single sporangium and breed true with the exception of types I and IV. These latter two organisms represent what may be conveniently termed reversible mutations: one type may revert to the other or it may give rise to a complex colony consisting of both types. This phenomenon has been described in a former paper.[2]

The five colonies illustrated in Plate XXXVII arose as mutations from the rhubarb *Phytophthora*. They are so unlike each other that no one would take them to be members of the same species and grown under identical conditions. It is probable that similar cases have been responsible for the description of many new species which after a careful investigation have failed to show a good specific value.

There are further differences in these five types. Figure 1 shows the character of the submerged mycelium and it can be seen that types I, II, and III have very irregular, gnarled, profusely branching, and bulbous hyphae, whereas those of types IV and V are fine and even.

Types I and II produce oogonia in abundance, type V not so readily, type IV sparingly, and type III none at all. Yet the presence or the abundance of oogonia are considered by the morphologists to be of very important taxonomic value.

[1] Published with the approval of the Director of West Virginia Experiment Station as scientific paper No. 21.

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Perhaps the most important features used by the morphologists in the classification of Phytophthoras are the size and the shape of sporangia. Whenever an investigator finds a Phytophthora strain which has sporangia differing a little from those of the described species, he is apt to think that mycology is about to receive another important addition to the list of new species. The micron, however, is not as efficient as it appears to most mycologists, as can be seen from the following experiment: Rhubarb plants

FIG. 1.—Submerged hyphae of the five types of rhubarb *Phytophthora*.

were inoculated with the five types of *Phytophthora* mentioned above. After the infection occurred, two hundred sporangia were measured from each of the five series. Since no sporangia are produced on the host under natural conditions, and since types III and V attack the host with great difficulty if at all, water cultures were tried and found satisfactory. Young rhubarb plants were transferred to Pfeffer's solution and inoculated. The moist condition induced the formation of profuse aerial hyphae upon the infected tissues and within two days large numbers of mature sporangia appeared. Several plants were used to furnish the sporangia of each type, and inoculated plants from two or more series were included in order to make the measurements more representative. Table 1 gives the summary of these measurements.

TABLE 1.—*Frequency distributions in percentages and constants in microns for length and width of 200 sporangia of five types of Phytophthora*

Class centers in microns	type I		type II		type III		type IV		type V	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
7.0	0	0	0	0	0	0	3	3	0	0
10.5	0	0	0	0	0	0	8	12	0	0
14.0	0	1	0	0	0	0	18	23	1	1
17.5	2	1	0	0	0	0	34	34	1	4
21.0	1	4	0	1	0	0	15	11	4	8
24.5	1	4	1	3	1	5	11	10	10	14
28.0	3	6	1	9	2	5	6	5	15	20
31.5	7	13	3	14	3	15	1	1	15	22
35.0	13	24	15	30	10	25	1	1	18	17
38.5	8	15	7	17	8	17	1	0	11	8
42.0	8	21	21	15	18	24	2	0	10	5
45.5	15	7	13	8	16	5	0	0	6	0
49.0	4	3	12	3	16	2	0	0	5	0
52.5	14	1	15	1	17	1	0	0	3	0
56.0	6	0	7	0	4	0	0	0	1	0
59.5	8	0	4	0	4	0	0	0	0	0
63.0	4	0	1	0	1	0	0	0	0	0
66.5	3	0	0	0	0	0	0	0	0	0
70.0	3	0	0	0	0	0	0	0	0	0
Mean size in microns	43.70	33.20	43.70	36.70	43.70	37.30	25.50	21.00	35.00	28.00
Median size in microns	45.56	35.31	42.81	35.35	47.91	35.53	17.92	17.59	34.88	30.06
Mode in microns	45.50	35.00	42.00	35.00	42.00	35.00	17.50	17.50	35.00	31.50

It can be seen that types I, II, and III manifest only small differences in the sizes of their sporangia. But note the sharp drop in spore size of type IV. Less evident differences have served as the basis of establishing many new species of fungi. In this case, however, no such practice can be tolerated because type IV is not fixed in its physiological or morphological habits and may revert at any time to type I. When such reversion occurs the mode for length of sporangia changes from about 18μ to 45.5μ. This reversion is continuous and independent of outside conditions. How can the morphological system classify such a fungus? Shall we say that this

same organism is one species at one time and another species at another time?

The sporangia of type V are also very small and more spherical as compared with the first three. On this basis alone it would undoubtedly be considered as a new species or else a species entirely different from *P. omnivora*.

It has been demonstrated previously that the size and shape of sporangia show wide variations under different environmental conditions; therefore it is only natural to expect that a change of host would induce certain clear-cut differences. In order to test this possibility some green pepper fruits were inoculated in the laboratory with the pure culture of type I of the rhubarb *Phytophthora*. Pepper was found to be a very good host and induced a profuse growth of the fungous mycelium; only a few sporangia formed, however, even after a long period of growth. But when a piece of the infected pepper with the attached mycelium was transferred to a covered glass dish containing a shallow layer of Pfeffer's solution, sporangia formed in great abundance within two days. The sizes of 400 of these sporangia taken from two lots of peppers are shown in the last four series of measurements given in table 2. The two lots of peppers were inoculated at different times. The environmental conditions in all of these experiments have been nearly the same and the temperature was held at approximately 23° C.

A difference of ten to twenty-one microns between the mode lengths, and seven to twelve microns in the mode width of sporangia of the same type of fungus when grown on rhubarb and pepper respectively, must seem rather disconcerting to those who have not hesitated to accept a margin of from three to five microns as sufficient in specific differentiations. Yet it readily can be seen that in the foregoing four measurements there is a fluctuating margin of nine microns in the lengths and about five microns in the widths.

It has been quite a vogue among some mycologists to classify the different types and sizes of spores on the percentage basis and to make it an important factor in identification work. The foregoing tables, however, do not encourage such a practice.

The mycelium which grew from infected peppers was used to inoculate another series of rhubarb plants. After this host was killed and the sporangia appeared, a number of measurements were made. It can be seen from the first two series of measurements in table 2 that the size of sporangia from the rhubarb host was noticeably increased (49 x 42 μ as against 45.5 x 35 μ) when the fungus was cultured on the pepper and the in-

TABLE 2.—*Frequency distributions in percentages and constants in microns for length and width of 400 sporangia of Phytophthora type I grown on rhubarb and on pepper*

Class centers in microns	On rhubarb		On rhubarb from pepper		On pepper Lot 1		On pepper Lot 2		On pepper Lot 3		On pepper Lot 4	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
7	0	0	0	0	0	0	0	0	0	0	0	0
10.5	0	0	0	0	0	0	0	0	0	0	0	0
14	0	1	0	0	0	2	0	0	0	5	0	0
17.5	2	1	0	1	5	9	3	9	10	14	6	10
21	1	4	0	4	11	21	11	14	10	16	13	23
24.5	1	4	2	8	13	15	15	21	12	19	17	23
28	3	6	5	3	16	26	12	15	14	15	13	13
31.5	7	13	2	4	15	14	12	21	12	14	9	10
35	13	24	3	15	13	10	16	11	12	10	13	12
38.5	8	15	4	15	11	2	12	8	9	4	10	6
42	8	21	7	30	9	1	11	1	8	2	8	1
45.5	15	7	7	10	2	0	3	0	5	1	2	2
49	4	3	23	5	3	0	2	0	3	0	4	0
52.5	14	1	20	5	2	0	3	0	3	0	3	0
56	6	0	14	0	0	0	0	0	0	0	1	0
59.5	8	0	8	0	0	0	0	0	0	0	1	0
63	4	0	5	0	0	0	0	0	0	0	0	0
66.5	3	0	0	0	0	0	0	0	0	0	0	0
70	3	0	0	0	0	0	0	0	0	0	0	0
Mean size in microns	43.70	33.20	43.75	35.00	35.00	28.00	35.00	29.70	37.30	29.70	38.40	31.40
Median size in microns	45.56	35.31	00'1₱	00'6₱	31.43	27.37	34.26	30.45	29.13	24.96	34.36	25.05
Mode in microns	45.50	35.00	49.00	42.00	28.00	28.00	35.00	24.50	28.00	24.50	24.50	22.75

oculated into rhubarb. This increase, however, is not considered as a carrying-over effect, but merely a normal fluctuation.

Neither can the shape of sporangia be considered a significant point in taxonomy. A glance at figure 2 dispels any illusion that one may have con-

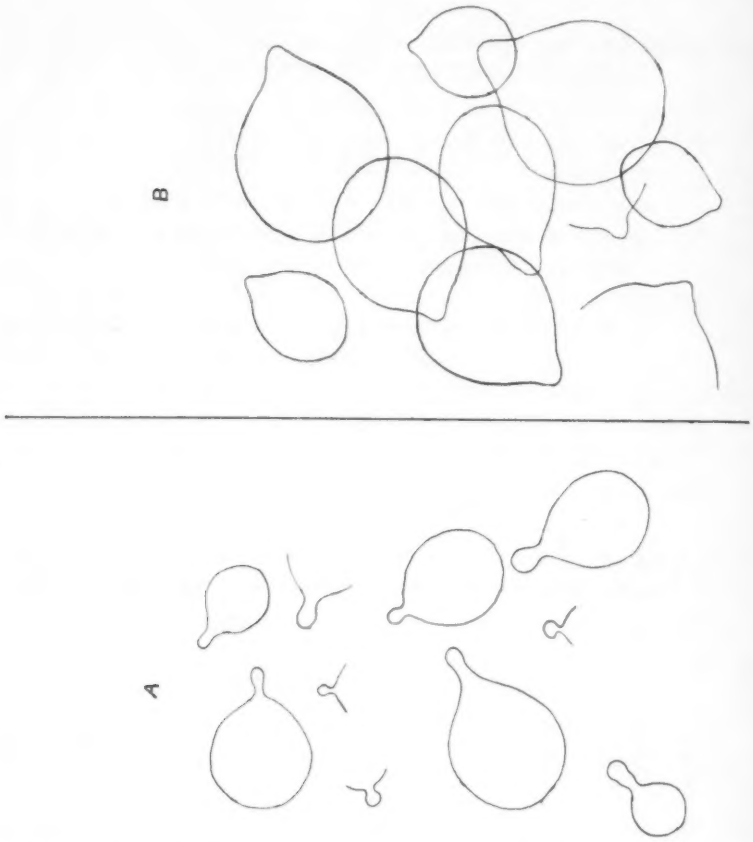

FIG. 2.—The relative sizes and shapes of sporangia of the rhubarb *Phytophthora* type I when grown on pepper fruit, A, and on rhubarb, B.

cerning the value of the shape of sporangia. In addition to the small size the sporangia from the pepper fruit usually possess very prominent papillae as contrasted with the much less pronounced papillae of the sporangia from rhubarb plants.

Morphologists have also considered the pathogenicity of an organism as an important and dependable character, but experiments conducted with the rhubarb *Phytophthora* and its mutations indicate that host relationships are no more reliable or significant, at least in this organism, than spore size.

Hundreds of rhubarb plants, young and old, were inoculated with the foregoing five types. All possible precautions were taken to eliminate errors. Disinfected seeds were grown in sterilized soil and the seedlings were later transplanted to steamed soil in the greenhouse and inoculated.

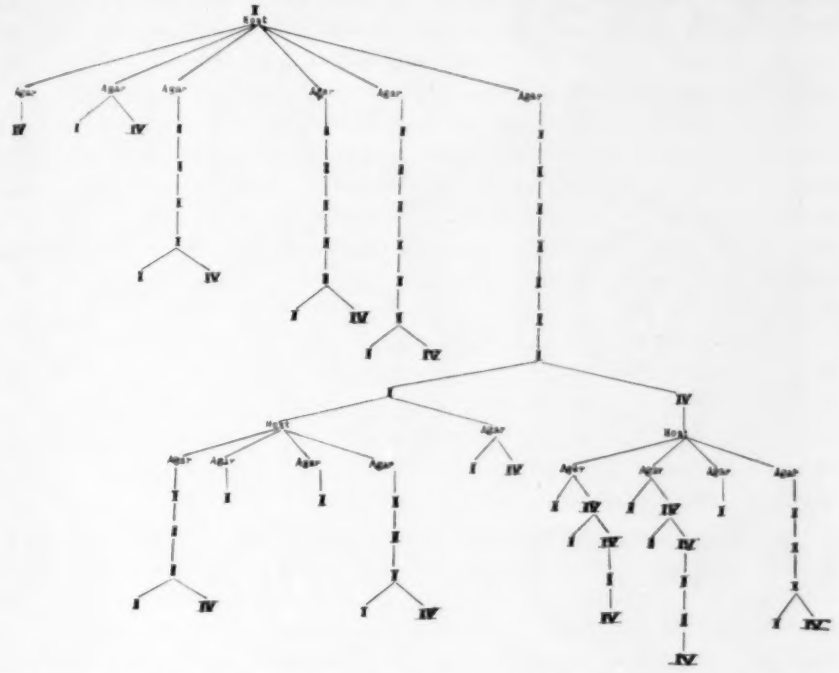

FIG. 3.—Chart illustrating the behavior of the rhubarb *Phytophthora* type I. The host was inoculated with a pure culture of type I; after the death of the plant six isolations were made on agar. Note the reversion, the splitting into two forms, the breeding true for a number of generations and then splitting and reverting again, thus illustrating a most remarkable plasticity and reversible mutations.

Numerous isolations were made from the infected plants to be certain that no other organism was a causal factor. These inoculation experiments were extended over nearly a year. A large number of full grown plants were used in addition to the seedlings and younger plants, and it has been concluded that types I, II, and IV are strongly pathogenic while types III and V can be eliminated as of no economic importance. Only very rarely did type III kill the host outright. Whenever an infection occurred it was confined to only one or two leaves and soon disappeared leaving no bad effect upon the host. Type V was unable to kill the host and only rarely was it able to attack a leaf or two. Even in water cultures where the host was weakened and the mycelium was stuffed inside of the host tissues, great

difficulty was experienced in inducing satisfactory infection. Christensen[3] has observed similar physiological specialization in the mutations of *Helminthosporium sativum*.

It has been seen that the nature of fungous colonies, the shape of the submerged hyphae, the presence or the absence of oogonia, the shape and the size of sporangia, and finally the pathogenicity of these organisms are not dependable factors, and if one is to follow such characters exclusively, confusing and even ridiculous situations may be expected to arise.

Studies conducted thus far and partially reported[4] indicate that a physiological scheme of classification where a great number of reactions are available can avoid such confusions in taxonomy because apparent specific differences disappear to such an extent that all of the foregoing five types, as well as a number of other supposedly distinct and authentic species and undescribed forms can be classified under one species group, namely *P. omnivora*.

It has been stated before that types I and IV, although appearing as very distinct forms, revert to each other or give rise to complex colonies. All attempts to control this by the manipulation of environmental factors have given negative results. It was hoped that perhaps the host would exert a stabilizing influence and fix the type, but figure 3, which shows the result of this attempt, dispels all such hope.

Reversible mutations are not uncommon; the writer has observed and isolated a great number of these not only in Phytophthoras but also in Fusaria. These phenomena play havoc with the present scheme of classification and they can not be ignored by ascribing them to environmental influences. Until some investigator is more successful in controlling such phenomena they will remain as taxonomic puzzles to be solved only by physiological methods.

SUMMARY

Work with some mutations of *Phytophthora omnivora* DeBary has indicated that the nature of fungous colonies in pure cultures, the shape of the hyphae, the presence or the absence of oogonia, the shape and the size of sporangia, and finally the ability to produce disease on a given host are not dependable factors in the taxonomy of the genus *Phytophthora*.

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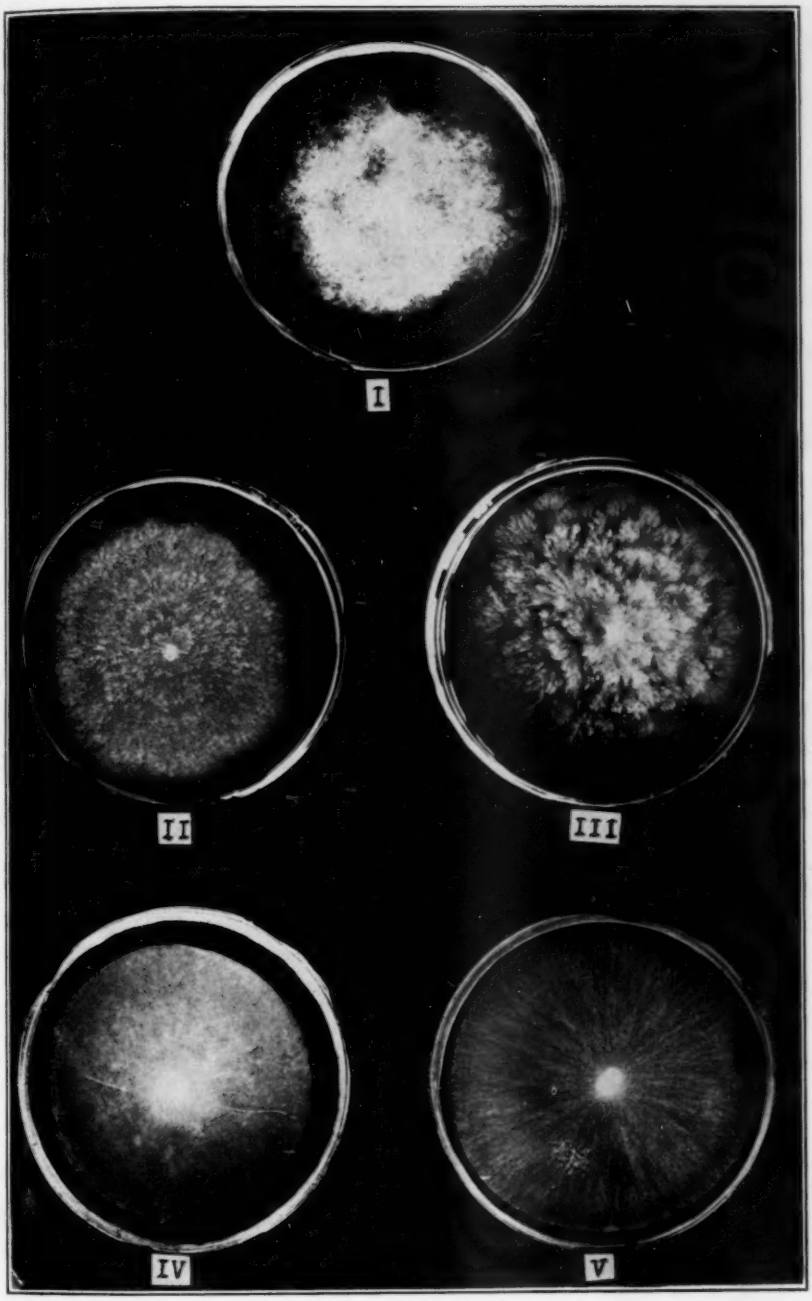
EXPLANATION OF PLATE XXXVII

Colony characters of the five types of the rhubarb *Phytophthora*. Type I is the original form which gave rise to the other four; it was grown from a single sporangium.

[3] Christensen, J. J. Physiologic specialization and mutation in *Helminthosporium sativum*. Phytopath. 12: 785–795. 1925.

[4] Leonian, *loc. cit.*


I
II
III
IV
V

HEREDITARY ABNORMALITIES RESEMBLING CERTAIN INFECTIOUS DISEASES IN BEANS

WALTER H. BURKHOLDER AND ALBERT S. MULLER

Two striking abnormalities of the bean plant, *Phaseolus vulgaris* L., have come under the observation of the writers during the past few years, each so similar to a common disease of this crop that some confusion was experienced before it was learned to distinguish them from the diseases they resembled. That they could not be classified as diseases was determined when it was found that fungi, bacteria, or infectious principles were not associated with them, but that they appeared to be transmitted only to the progeny of affected plants, and then as recessive characters. Genetical literature is full of cases of abnormalities in plants being inherited, but in very few instances have these abnormalities been mistaken for diseases. Such a one, however, is reported by Dr. R. A. Emerson[1] in the case of blotch leaf in maize. The appearance of the blotch on this plant suggests a fungous or a bacterial disease, but the character is inherited as a recessive, as he has shown. The two abnormalities found in beans and discussed in this article are: first, a leaf character similar in appearance to the symptoms on leaves caused by the mosaic disease; and, second, a seedling wilt, for a time thought to be due to a fungous or bacterial infection.

THE PSEUDO-MOSAIC

The term *pseudo-mosaic* was applied to this character of the bean leaf for convenience, to distinguish it from the true mosaic of this plant. In appearance the pseudo-mosaic differs from the true disease in the following characters. The leaves are narrower, longer, and have more or less of a tendency to twist. There is also a more striking difference between the lighter and darker green areas. The dark areas are fairly normal in appearance, and not so raised or cupped as in the true mosaic. The light areas, on the other hand, are frequently yellowish, and occur at times as a series of streaks on the leaves. If examined closely, one can observe a fasciation or an anastomosing of the veins as the nucleus of the lighter areas. The increase of the vascular tissue is, no doubt, the main cause of the yellow appearance in these areas, but there also appears to be less chlorophyll in the surrounding cells.

[1] Emerson, R. A. The inheritance of blotch leaf in maize. N. Y. (Cornell) Agr. Exp. Sta. Mem. **70**: 1–16. 1923.

The true mosaic frequently changes the type of growth of the bean plant and gives it a bushy appearance with spindly runners. On the other hand, the effect of pseudo-mosaic is limited to the leaves entirely. The yield is not affected by this abnormality, but this is no criterion for dis-

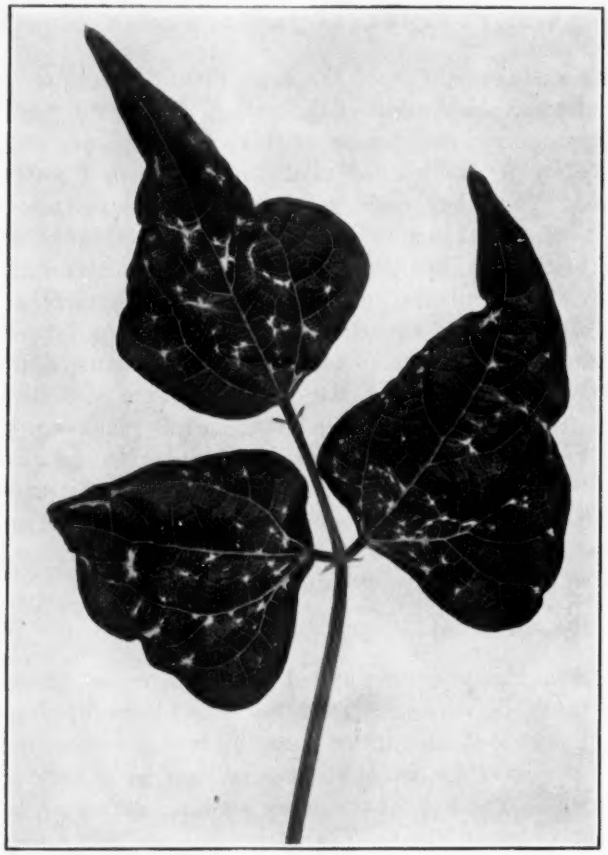


FIG. 1. The pseudomosaic: an hereditary abnormality of bean leaves. The photograph was taken with a ray filter which caused the anastomosed veins to stand out very clearly.

tinguishing the two, since certain varieties of beans will produce a good yield when practically all their leaves show unmistakable symptoms of the true mosaic.

A chart obtained by the Department of Plant Pathology at Cornell University from the U. S. Bureau of Plant Industry, labelled chart 8 Bean Mosaic, CT. 3304, gives in colors very distinctly these two types of leaf

characters, of the true mosaic, and of the pseudo-mosaic. In examining these charts, one may notice that the two top leaves show symptoms of the mosaic disease. The two lower leaves in appearance are typical examples of the pseudo-mosaic dealt with in this paper. Whether the originals of these two lower pictures were a variation of the true mosaic, or a different mosaic, or pseudo-mosaic, it is impossible to state.

The pseudo-mosaic was first brought to our attention in the summer of 1919, when one selection of beans (3292) grown on the experimental plots at Perry, New York, showed 100 per cent pseudo-mosaic. This selection had come from a cross between a White Marrow and a Flat Marrow, and was being grown for its resistance to the root rot caused by *Fusarium martii* f. sp. *phaseoli* Burkh. At that time, while we had noted the two types of mosaic in the bean fields of New York State, we were inclined to consider them variations of the same disease. However, since the type of mosaic on this selection was the uncommon type and there was 100 per cent infection, it was looked upon with some misgivings. None of our selections had ever shown an approach to 100 per cent through natural infection.

Consequently, some inoculation experiments were conducted with this type of mosaic which we now term pseudo-mosaic. At that time on the experimental plots we had growing approximately 100 varieties of beans. Ten plants of each variety were selected and inoculated by rubbing an abnormal leaf over a leaf on one of the healthy plants, care being taken that the epidermal tissues of the healthy leaf were broken in the process. While a small percentage of mosaic developed on the inoculated plants, in no case was the percentage higher than on the remainder of the uninoculated plants. The mosaic which did develop was of the common type and was probably brought in by insects. The indication from these inoculations was that the pseudo-mosaic was not infectious, and further experiments in the greenhouse seemed to corroborate this. It was also noticed that this new type of mosaic had no effect upon the yield. Selection 3292 bore heavily that season; the seed was saved and planted in the greenhouse the following winter and in the field during the summer of 1920. All seeds produced plants having this peculiar leaf character. Seeds from a bean plant affected with the true mosaic seldom give rise to 50 per cent diseased plants. It should be stated here that the pseudo-mosaic character in the greenhouse is not very distinct at times. In the gardens, too, it may vary considerably in intensity of character from year to year. Late in the season the intensity of the abnormality is greater as a rule, and has more the appearance of the mosaic disease. From this it would appear that the character is affected by the environment, a fact which is also true of the symptoms of common mosaic on the leaves.

This problem was not followed up immediately, but in the winter of 1923 a number of crosses were made between selection 3292 and the Robust

Pea bean. Reciprocal crosses were made. In all, 19 pods bearing 53 seeds were secured from these crosses. These seeds were planted during the summer of 1923 and harvested the first of October. Forty-three of the F_1 plants survived and bore seed, but in no case did the pseudo-mosaic character appear on any of the leaves, a fact which indicated that the character, if inherited, was recessive.

During the summer of 1924 the seeds from these F_1 generation hybrids were planted at Ithaca, New York, in a garden removed from all other bean plants; and data were taken on the F_2 individuals. Counts were taken on 34 families containing 1280 individuals. Of these, 75 showed the abnormal leaf character, and 1205 appeared normal. This is quite a close approximation to a 15:1 ratio (1200–80). In no case was any true mosaic observed to complicate the taking of the above observations. Sufficient data seem to have been presented to show that the pseudo-mosaic is not an infectious disease but an abnormality, and one which is hereditary. Good genetical data as to the manner of its inheritance, however, are lacking. To check up the 15:1 ratio obtained in the F_2 generation, it would have been desirable to grow the F_3 plants. This was impossible, however, since the F_2's were late in maturing and were killed by frosts. A further objection might arise as to the use of the Robust Pea bean for one of the parents, when it was known that this bean is resistant to the true mosaic. However, by the use of this variety the chances of a true mosaic entering the experiment are lessened. And, furthermore, McRostie[2] has shown that susceptibility to the mosaic disease is partially dominant, a ratio of 9:7 being obtained in the second generation. Artificial inoculations were necessary in his experiments.

In conclusion we wish to state that, while we hold the pseudo-mosaic to be only an abnormality, similar symptoms on the leaves of beans might be due to a type of mosaic not frequently encountered in the bean fields of New York State.

THE SEEDLING WILT

This abnormality was first brought to our attention by Dr. G. P. McRostie of the Department of Plant Breeding at Cornell University. Dr. McRostie observed, while working on the genetics of the resistance in beans to *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. et C., f. sp. *alpha* Barrus, that a number of his F_2 seedlings died. The crosses were between the Well's Red Kidney and the Robust Pea bean. The trouble outwardly appeared to be an infectious disease of some kind. A great many of the dying seedlings were examined and, although no associated organism was

[2] McRostie, G. P. Inheritance of disease resistance in the common bean. Jour. Amer. Soc. Agron. 13: 15–32. 1921.

found, the writers were inclined to consider it a disease. In appearance and in progress of the trouble the abnormality was as follows. There was no retardation in the germination of the seeds and, unless carefully examined, what appeared to be a healthy seedling would be sent forth. In many of the abnormal seedlings the green was slightly off color. This was

FIG. 2. Hereditary seedling wilt of beans.

hardly noticeable, however, except in contrast with healthy plants. From this stage on, the plants sickened and died. There was no rapid flagging of the leaves as is frequently the case with certain bacterial infections, but otherwise the symptoms were not unlike those produced by certain organisms. The primary leaves became pale and at times had a bronze appearance. They curled somewhat and frequently dropped off. Further growth was seldom made, although, in a few cases, where care was taken of the plants, a few more leaves were added and occasionally a flowerbud was formed before the plant finally died.

In examining wilted individuals, no lesions were found upon the plants; the roots were especially clean. The vascular system too, appeared normal, and search for bacteria or fungi was of no avail. A great many plantings of stems, roots and leaves from these affected plants were made in agar in petri dishes, but no pathogene was isolated. Seeds planted in pots which had had wilted seedlings in them produced healthy plants, although the former wilted seedlings were cut up and placed in the soil. From such experiments it became evident that the trouble was not at all, or at least not readily, communicable.

A clearer understanding of the matter was arrived at when it was observed that the seedling wilt only occurred in bean hybrids, including and after the F_2 generation. Furthermore, it did not occur in all hybrids. It was very frequent in crosses between the Well's Red Kidney bean and the Robust Pea bean, but it never was found in crosses between the Well's Red Kidneys and the White Marrow.

During the season of 1922 a great many individuals of the second generation of hybrids were grown, involving crosses among ten different varieties of beans. The work was being done in connection with some breeding work for resistance to the bacterial blight of beans. Among these seedlings a great many wilted plants appeared, so it was considered worth while to determine the ratios of the wilted to the healthy plants and what varieties of beans were involved. Crosses had been made among the following varieties: Baldwin Wonder Wax, California Wonder Pea, Giant Stringless Green Pod, Keeney's Rustless Golden Wax, London Horticultural, Robust Pea, Refugee Green Pod, Scotia, Flat Marrow, and the Well's Red Kidney. The seedling wilt appeared only in those hybrids of which one of the parents was the California Wonder Pea bean, the Robust Pea bean or the London Horticultural bean, but did not always occur under these circumstances. Families which were entirely free from the wilting seedlings appeared, on the whole, to be composed of healthier individuals than those in which this abnormality did occur. This was also observed in the F_3 generation, but no careful measurements were taken on this point. In the F_2 generation of 16 families containing 1795 individuals, 119 wilting seedlings were found, and 1675 normal ones. A 15:1 ratio which this approximates would be 112 to 1683. It would appear from this that two factors were involved, and, when both were absent from the same plant, that individual would be unable to grow beyond the seedling stage. Data taken on the F_3 generation, however, did not fully confirm this.

The seeds which produced the F_3 generation were two years old before planting, and the number which was planted was not recorded. Consequently the ratios obtained were based only on the seedlings, and the ungerminated seeds were not considered. These might have changed the

ratios. As it was, over one-half of the families threw no wilting seedlings, and those that did again gave a ratio of 15:1. The 3:1 ratios which were expected did not appear. The above data are by no means complete, especially from the genetical aspect, although we feel that they do show the trouble to be of an hereditary nature.

SUMMARY

Descriptions are given of two abnormalities of the bean plant, *Phaseolus vulgaris* L.: one a leaf character resembling a mosaic, and the other a seedling wilt. Failure to find a causal organism or an infectious principle led to a genetical study of the two characters. Data concerning their mode of inheritance indicate that both are recessive characters and that two factors are involved in each case.

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THE TEACHING OF ELEMENTARY PLANT PATHOLOGY[1]

L. R. Hesler

The interest which is being manifested in the teaching of plant pathology is encouraging. But no matter where a quickened enthusiasm may lead, we must focus our attention rather steadily upon three vital aspects: the subject, the teacher, and those who are taught. Fundamentally plant pathology is the same wherever and however it is presented. The method used by the teacher is important, and undoubtedly varies widely in its details. But, whatever the method, it is more pertinent to inquire into the philosophy and broad aims of the teacher than to become lost in mere method. The interest of the one who teaches should be in the student as well as in the subject; the teacher should be concerned not so much in whether the student will make a living but how he will think and live.

The course in elementary plant pathology at the University of Tennessee is organized on the crop-basis. It is believed that this plan is at present well suited to the needs of the students taking the course, although the plan, both in general and in detail, is ever subject to new developments in plant pathology and in other fields of science. The general trend of education, it is hoped, will also exert an influence on the form of the course here considered. It is always wise that the teacher shall seek new points of view to which he may shift in order to lessen the danger that the landscape may become worn out by familiarity.

All students registered for the Bachelor of Science in Agriculture degree are required to take the course in the second semester of the sophomore year. (Recent action of the faculty places the course in the fall term of the junior year.) Moreover, all students in the two-year agricultural course are held for plant pathology. Further, the course is designated as an elective for liberal arts students, although with few exceptions only agricultural students take it.

Prerequisite botany consists of a four-hour semester course in fundamental plant studies, dealing largely with elementary plant physiology, with sufficient anatomy included to assist in an understanding of functions. Agricultural students are required to take this course in the freshman year. In the first half of the sophomore year, four semester-hours of general bacteriology are required. The elements of physiological and structural botany and bacteriology are, then, courses prerequisite to our first

[1] Invitation paper read before the American Phytopathological Society, Kansas City, December, 1925.

course in plant pathology. At the beginning of the first term of the junior year these students begin the study of plant diseases.

The course at Tennessee is a four-hour subject. The meetings consist of two lectures of one hour each and two laboratory periods of two hours each. Although most of the subjects in the agricultural curriculum are three hour courses, the curriculum committee has made elementary plant pathology, along with the more general sciences, a four-hour course.

At the outset, let us not overemphasize the importance of the method of classifying the subject-matter. There are perhaps several good plans on which a course in elementary plant pathology might be built, but undoubtedly much more depends on the teacher than on the plan of organization. It is contended, however, that some well-laid plan is necessary. But it should not be forgotten that the student may fail to appreciate the elegance of the plan; what he sees, after all, is not the plan but the teacher.

The crop-basis plan of presentation characterizes the course in elementary plant pathology at the University of Tennessee. With minor exceptions, the diseases of a given crop are studied in sequence, although this does not mean that no emphasis is given to other important aspects, such as etiology, which might serve as the basis for classification of subject-matter. In our procedure the emphasis is placed wherever it seems possible to bring out a principle. In some cases the stress may be put on lesion-type, in other instances on variety of symptoms exhibited, and in all cases the causal factor is examined as fully as possible. The primary division of the subject-matter, however, is the crop-basis.

An attempt to point out the advantages of such a plan raises the question of the objective. The aim should depend somewhat on what the student does after having had the course. The needs of the student rather than the whims of the teacher should be considered. The aim should also be determined, in part, by what best fits into the general curriculum of the college. An ideal, perhaps, is that the course shall attempt to reveal the relationship of plant pathology, a specialized branch of science, to knowledge as a whole. Finally, whatever may be the ultimate aims, the more immediate goal is to train the student, by means of plant pathology, in methods of scientific observation, thinking, and reasoning. In striving for this aim one is certain to teach something of plant pathology as a factor in agriculture and to enlist the sympathy and interest of students in our worthy field of study. The question then arises as to whether the method employed accomplishes these objectives.

In defense of the crop-basis method of organization, it may be said that a farmer is in the habit of grouping his problems by crops; crop production is his occupation. It is natural, then, that the agriculturist will think of his problems from the stand-point of the crop affected. He is interested

in the remedy. Remedial measures at times are directed against more than one pest affecting a crop. The important diseases of a crop can be arranged in seasonal sequence and a definite control-program can be offered.

Experience indicates that the student will return to the farm, either as a crop grower or as a rural leader. Therefore the beginning course in plant pathology should train him to recognize disease, to diagnose the more common diseases, and to help him understand the cause of disease. Such a training should lend support to his intelligent pursuit of any plant disease problem that may arise. If the problem is simple, he may solve it himself; if complicated, he will recognize that fact. In presenting the subject on a crop-basis plan, the plant disease problem is hitched to something already familiar to the student. On a basis of lesion-type or a causal agent, it is more difficult to make ready contact with the student's previous knowledge and experience. Suppose we divide plant diseases into necrotic, hyperplastic, etc., and approach the subject through these groups; or suppose we classify diseases on a basis of the causal factor, bacterial, fungous (and further, phycomycetous, ascomycetous, etc.) and then lead the student to plant pathology for his assimilation. In either case there is little in the student's previous experience that enables him to tie on to plant pathology, which for him is a new subject. No farmer ever started out to control bacterial diseases on the farm; but he does have interest in a program dealing with the diseases of a single crop. At the same time, however, there is no intention of denying the student a knowledge of the causal agent—a knowledge of which is essential to a thorough understanding of control, and is vital to an appreciation of the problem of disease in plants. In fact, the causal factor in each case is studied as fully as conditions will permit. Special attention is devoted to details of morphology and life-history of parasites. The final stress is then placed on those features of cycle and structure which bear on control. It is not that we minimize etiology; on the contrary we emphasize it. The general approach, however, is through crop groups. Of course, there are certain disease-types which group themselves somewhat naturally on an etiologic basis. For example, the mildews, smuts, and rusts constitute familiar groupings even in the lay mind, so that each of these types may well be treated together in an elementary course in plant pathology.

The course offered at Tennessee is general, covering the diseases of various crop-types. Chief emphasis is given to the more common diseases. It is felt that it is better to study thoroughly a few selected types than to attempt a review of a large number of known diseases of a given crop. With few exceptions, two laboratory periods are given to the study of a specific disease. Certain important diseases are necessarily omitted in such a scheme, but some of these are brought in for demonstration when the

control program for the disease of a given crop is under consideration.

The diseases which are selected for detailed study are those of chief importance in Tennessee. Some discussion is presented regarding certain diseases of national or even international importance in order to broaden the view of the student relative to the larger problems in plant pathology. I refer to such troubles as chestnut blight, citrus canker, white pine blister rust, potato wart and others.

The material actually employed in the laboratory exercises may be classified as fresh, dried, pickled; illustrations, cultures, and prepared slides. Dried and pickled materials are used in practically every study. In nearly every exercise, fresh materials are made available. Free use is made of illustrations; figures in books and bulletins, photographs—these are regarded as proper laboratory material. Sets of publications in particular, as far as available, are on file for the student's use as the need arises. Pure cultures of parasites are used wherever possible. The student is apparently more definitely impressed with the existence of an organism if it can be seen growing in mass separate from its host. Elementary spore-germination studies are made with the peach brown-rot fungus. This exercise is inserted in order that the student may better visualize incubation phenomena. Prepared slides are provided but to a limited extent and only after the student has knowledge of the source of the mount. These slides are used largely to facilitate study of details of fruiting body structure, and to aid in understanding pathological changes in diseased tissues (as in peach leaf curl). An abundance and variety of materials are aimed at, on the assumption that visual learning is best. Diagnosis, if it is to be learned at all in such a course, is learned from a large assortment of materials. Lantern slides are not available; when wisely used, they are doubtless of value.

Brief notice of the nature of laboratory procedure may be pertinent. After preliminary explanations are made, a difinite assignment of the exercise is announced. Time in the laboratory is largely spent by the student in a problem study of symptoms and in the more essential aspects of etiology. Drawings of chief features of the disease and parasite are made by the students. No time is given by the students to isolation of organisms nor to pure culture preparation; but enough practice in inoculation work is included to acquaint him with the general technique, the importance of, and the fact of, pathogenicity. Two field trips are on the program: one assumes the nature of a survey of the University farm, including cereals and fruit; the other of a vegetable-growing section nearby.

Written work is a valuable adjunct to the laboratory exercise. At the conclusion of each study, certain topics or questions are set forth. The student, with selected references to literature, writes on the topics as-

signed. The written work, together with the drawings made in the laboratory, constitutes the student's report on a unit of work.

One special report is required during the semester. This report is in the form of a longer paper, 1500 to 4000 words, which is compiled from the literature. The value in having the student go to the original sources is recognized. How literature originates, how to find it, its magnitude, its value, its use especially in so far as training is gained in sorting, writing, citing, editing, and the like—all these questions can be given attention in an exercise such as this. The student is asked to choose a subject, one pertaining to a plant disease which has not been included in the laboratory program; on this topic he is to write the paper. He is taught the nature and use of the Experiment Station Record as a source of references to literature. Later, other sources are introduced. When a reasonable number of references is accumulated, the student goes to the original publication, and the subject matter contained therein is abstracted. In making the abstract, the material is sorted, and the notes are made under headings definitely arranged in an outline. Abstracts are finally brought together and used in compiling the paper. The additional information on plant diseases and the peculiar training afforded by this type of exercise seem to justify the undertaking, alike in the eyes of instructor and students.

Thus the laboratory work is largely descriptive plant pathology. This is intended. The laboratory studies precede the lectures; this arrangement is also intentional. The lecture work deals to no little degree with analytical plant pathology: the "structure" of phenomena, the philosophy of the various aspects and recommendations, the inter-relationship of technical knowledge and practical application, the exact use and meaning of terms, and the interpretation of the more obvious truths. The lecture aims, then, at a ground-work which is necessary to an understanding of control-recommendations and practices. Usually most of the lecture hour is given over to a discussion of those features of a disease which are more definitely related to control; as a rule, however, the minimum time is actually devoted to details of the control measures themselves. An understanding of the principles on which control practices are based would seem more desirable than an extended discourse on methods of control. The methods, at least in detail, may change within a short time, whereas principles change more slowly. In each lecture, one point is stressed more than others; a theme properly selected and emphasized gives the student a frame-work to which subject-matter detail can be attached. In every instance, too, the important points regarding the various aspects of the disease are covered; these aspects are taken up in a definite order for each disease discussed and is practically uniform for all lectures.

No text-book is required. References to the best available literature are given out both at the lecture and in the laboratory. In some cases, where the

available literature is not suitable for student use, the lecture subject-matter is mimeographed and is distributed subsequent to the lecture. In such instances, as in many others, the students are tacitly advised against taking notes. This suggested procedure is based on the theory that taking notes results in a divided attention. I am inclined to the opinion that lectures and note-taking are over-done.

The so-called intelligence-test type of examination has been in use at Tennessee in the Department of Botany for a few years. The examination is divided into parts, or tests, as for example: true-false, selection, completion, matching, labelling drawings, and sometimes spelling. The advantages and disadvantages are probably known to everyone interested. While this kind of examination has proved useful and popular in our course, yet this type is not used to the exclusion of others. A few of the usual types of examinations, and frequent short oral or written quizzes are given. A final examination is given, but the emphasis is placed on daily work rather than on such a test.

In presenting any subject in a college curriculum, we should not lose sight of the student. The interest of the teacher should be in the student as well as in the subject. Sympathetic treatment of the student has a positive place in any course. It is advantageous to gain the student's point of view and criticisms; it has been said that the good teacher does not refuse to learn from his students. It is well to aim at the development of the maximum in the student's personality and character, but for the subject the aim should be to give the student only the optimum. The teacher's knowledge of his students should rate as high as his knowledge of the subject. Attempt should be made to give to the student "the spirit of learning, not learning itself"; and consideration should be given to the development of capacity, rather than to the stuffing of an already quite limited intellectual store-house.

Principles should be emphasized. Of course many facts are taught; it must be so! Facts must be stressed, but the final emphasis is put upon the principle which is synthesized out of the facts. Few rule-of-thumb formulae are taught; these can be had without taking the course. It is not enough to shower the student with an array of facts; principles must be developed. Nor is it sufficient merely to develop principles; these must be stated and restated.

The final objective is "not learning but rather discipline and enlightenment." We have no right merely to exploit the student to satisfy a hobby, but we should employ plant pathology to widen his vision; and, finally, we should use the course so that, along with other courses in the curriculum, it will function to develop the student—to increase capacity, discipline his mind, his habits, his point of view, reflections, discriminations, insight, perception.

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SOME POINTS OF VIEW ON THE PLANT VIRUS PROBLEM[1]

JAMES JOHNSON

There are ardent supporters of most, if not all, of the conceivable theories concerning the casual agency of virus diseases. This lack of agreement seems to be largely the result of the chaotic state of the knowledge of details involved in the separate phases of the problem. It is probably quite within the power of present research technique to determine these details definitely. While fundamentally important progress has been made since the recognition of the first virus disease, the data and conclusions have unfortunately been so conflicting as to point in no general direction. The difficulties have been, and apparently will be, increased in the future by the fact that nomenclature is based largely on symptomatology. While we have in general a fairly distinct set of symptoms for virus diseases, these are limited usually to chlorosis, mottling, malformation, necrosis and stunting, with intergradations which soon render impossible the selection of valuable descriptive names for the disease. It may soon be advisable, therefore, to abandon the attempt to describe a virus by the disease which it causes in favor of some other system more comparable to that used in the classification of bacteria and certain fungi.

SPECIFICITY OF VIRUSES

Transmission experiments and symptomatology have demonstrated that, in the potato virus group, a dozen or more apparently distinct viruses exist. The question of specificity of these viruses has been determined largely through symptomatology and through inter-varietal inoculations. While it has been reported by some workers that certain of these potato virus diseases are transmissible to other solanaceous plants, one is led to wonder if the viruses in question actually were transmitted, in view of recent results obtained in transmitting viruses with equal ease from apparently healthy and diseased potatoes to tobacco. In any case, the majority of the characteristic potato virus diseases do not appear to be readily, if at all, transmissible to certain other closely related plants. A similarly restricted host range probably exists in the case of certain other groups of viruses.

[1] Invitation paper read before the joint session of the American Phytopathological Society and the American Potato Association, Kansas City, Dec. 30, 1925. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, and the Chief of the Bureau of Plant Industry, United States Department of Agriculture.

On the other hand, Elmer's recent work would indicate that the virus employed was not specific as to species, genera, families or orders. Fifteen inter-family and eleven inter-order transmissions are reported, presumably on the assumption that one virus was used in all cases. A year ago only one virus disease of tobacco was recognized in our laboratory. At present we recognize nine or ten distinct viruses, if we include those secured from the potato. Symptoms, differential hosts, and other characteristics plainly point to the conclusion that these viruses are distinct, though admittedly not easily differentiated in some cases. The ordinary tobacco mosaic has probably not been transmitted outside of the solanaceous family. A specific virus may in some cases, however, pass directly from one family to another, as in the case of cucumber mosaic to tobacco.

Elmer probably was not working with a single virus but with several viruses, since he states that infected plants employed as sources of inoculum were obtained from widely separated localities, a fact which greatly reduced the chance that any specific cosmopolitan virus was used. His results are nevertheless very suggestive in the interesting field of virus transmission and host range. To be of most value, however, his experiments should be repeated with the question of virus specificity more clearly in mind; and future host range studies should take this consideration into account.

COMBINATIONS OF VIRUSES

It has been believed for some time that various distinct viruses may coexist in individual plants, especially in the potato. The potato viruses and their host range are probably not so well adapted to a study of combinations of viruses as are many of those which attack tobacco and other solanaceous plants. Certain combinations of viruses are apparently much more likely to produce a malignant disease than a single virus. With nine or ten viruses affecting as many or more host species, there is ample room for studying this interesting relationship. The separation of the viruses which are purposely combined, or the study of possible combinations found in nature, often is quite feasible by the use of differential hosts and other methods of separation such as aging *in vitro* and the selective killing action of heat and chemicals. Cucumber mosaic and tobacco mosaic in the same inoculum should be separable, for instance, by inoculating to pokeweed to get the cucumber mosaic free from tobacco mosaic. On the other hand, by permitting the inoculum to age for two or more days outside the living host, the tobacco virus should be secured free from the cucumber mosaic virus.

INSECT RELATIONS

The present status of our knowledge of the fundamental relation of insects to certain virus diseases is confusing and is likely to remain as a

barrier to clear thinking on the virus problem until clarified. The limited evidence which exists indicating that certain insects play a necessary or fundamental part in the life cycle of the virus concerned should be extended and corroborated if possible. Experiments indicating that an infectious disease can be transmitted only by insects or by grafting need to be performed with great care in respect to method of inoculation, the source of the inoculum, the condition of the host, the environment and other circumstances in order to be wholly convincing.

In the meantime, however, these insect relations should not be permitted to confuse us in regard to the majority of our more typical filterable viruses, for which we can be reasonably certain that insects are not essential, and that the part played by them is in all probability purely mechanical.

From an economic point of view it seems generally accepted that insects are largely, if not entirely, responsible for the occurrence of epiphytotics of most of the virus diseases. Following several years of observation and considerable experimental work, we have yet to be convinced that insects play a major role in the occurrence of epiphytotics of tobacco mosaic, although admittedly no good single substitute which is generally applicable as an explanation has been definitely discovered. Transmission through soil by the various means of contact and by storms may be much more common than generally supposed.

ENVIRONMENT RELATIONS

The relation of environment to virus diseases will require much more investigation than has been accorded to it up to this time to be adequately understood. Virus diseases are radically different from most parasitic diseases in that moisture does not play an important part in infection. This may be accounted for by the fact that infection occurs largely through wounds and that the virus almost immediately enters into close association with the surrounding protoplasm. The virus must either grow throughout the plant from the point of infection or be carried through it by normal physiological processes. Judging by its rapid systemic invasion, it is likely that its multiplication and diffusion occur simultaneously. Temperature is perhaps the only factor to which the virus diseases studied are particularly sensitive, but it is a disputed question whether this influence is exerted upon the virus directly or upon the host plant and hence indirectly on the virus. The expression of potato mosaic is most typical and marked at comparatively low temperatures (15° to 20° C.). Above 25° C. the symptoms are masked to a striking degree, not only on the newly formed leaves but also on older leaves previously showing distinct mottling. Furthermore it does not require a continued high temperature to bring about

this result, as shown in our laboratory by Tompkins; up to 80 per cent of the time may be spent at the low temperatures with a similar, though delayed, final result. The masking of symptoms already present seems most easily interpreted on the basis that the virus is reduced in activity in some manner by the high temperatures. While it is generally recognized by all workers with mosaic that the optimum temperature for the vigorous development of the host is the optimum for the shortest incubation period, this does not entirely explain the symptomatology in the potato. The potato plant may develop more rapidly at 25° C. than at 15° C.; yet the symptoms of mosaic as they develop from the tuber will be much more marked at 15° C. than at 25° C. On the other hand, if healthy young potato plants are inoculated with the mosaic virus, the plants placed at 25° C. for some days will show symptoms much earlier than plants kept constantly at 15° C. The virus apparently is not attenuated by the higher temperatures. In other words, a temperature favorable for masking is at the same time near the optimal temperature for infection as measured by the length of the incubation period. Again, if one is working with two or more different viruses on the same host in constant temperature chambers, a given temperature may be considerably more favorable for infection by one virus than by the other; or, on the other hand, a given temperature may mask one virus disease and not the other. Consequently the influence of temperature probably is more significant than a simple influence on host development.

SYMPTOMLESS CARRIERS

The possibility of plants carrying a virus without ever being capable of expressing any symptoms of the infection is an interesting and important problem. If this is actually true, it indicates a peculiar physiological relationship in regard to the nutrition and reproduction of the virus. In this connection, from a pathological standpoint a fundamental difference exists between masking and symptomless carriers. In the case of masking, it is necessary to assume only that the virus is in a dormant condition, or, as is more likely, the host is not in a physiological or morphological condition satisfactory for the expression of symptoms. In the case of symptomless carriers the virus must be assumed to be actively reproducing itself at the expense of the host without in any way appreciably affecting its appearance. However, recent results lead us seriously to question the existence of the reported cases of symptomless carriers. Certainly the original case of *Nicotiana glauca* cannot be left in this category, since this host may be markedly stunted by tobacco mosaic and strikingly malformed by cucumber mosaic. The cases in *Physalis*, as reported by Nishimura and by Elmer, are usually regarded as typical examples of symptomless carriers. Our experi-

ments with these species recently have shown that symptoms can be obtained under favorable environmental conditions. As I have pointed out in a recent article, however, the potato may eventually prove to be an excellent example of a symptomless carrier of viruses, since apparently healthy plants appear to be commonly infected. On the other hand, certain plants like *Nicotiana glutinosa* and the potato, which may be seriously affected locally by a virus, do not appear to carry the virus systemically.

PROPERTIES OF THE VIRUSES

This fertile field for investigation is too large to venture far for present purposes. It may be recalled, however, that tobacco mosaic is comparatively resistant to aging, putrefaction, desiccation, heat, and chemicals. Cucumber mosaic on tobacco produces symptoms so similar that the fact that these viruses are different has escaped detection for years. The main properties of the cucumber virus, however, apparently remain essentially the same in tobacco as in the cucumber. As a source of inoculum for mosaic Elmer recommends the use of vigorously growing parts of infected plants. Our experience with the tobacco mosaic virus in overwintering studies has shown that virus harbored on old boards or rags for a period of two years is practically as effective when extracted from these materials as inoculum extracted from green plants. Elmer, however, may have been using the cucumber mosaic virus or a similar virus, in which case his conclusion is probably correct, owing to the comparative sensitiveness of some viruses to adverse conditions. In this connection it may be mentioned that the host plant itself may have a marked influence upon the value of the virus for inoculum purposes. Pokeweed, for instance, is in our experience a poor source of cucumber mosaic virus for inoculation to certain unrelated plants as compared with tobacco. This may explain in part the apparent value of bridging hosts in some cases, rather than any actual change in the nature or properties of the viruses.

While tobacco mosaic and other viruses are surprisingly resistant to certain toxic chemicals, they may be often readily destroyed by such milder forms of chemical activity as represented by the action of other living organisms or by enzymes. For example, the virus is readily destroyed in moist soil as compared with dry or sterile soil. In slowly curing tobacco (presumably an enzymatic process) the virus may be fairly rapidly inactivated as compared with tobacco which is rapidly dried or kept as a liquid extract.

SIZE OF PARTICLES

There still seems to exist a remarkable difference in opinion as to the probable size of the particles concerned in filtrable viruses. While it is

generally recognized that fine grades of porcelain filters will prevent the passage of a mosaic virus and that coarser grades will permit the virus to pass, at the same time yielding a sterile filtrate, it is yet maintained by some that certain microscopic protozoa and bacteria are filter passers and that they are consequently not necessarily excluded from the category of probable causal agents. Others believe that ultramicroscopic fragments of these organisms, capable of acting as reproductive bodies, may pass extremely fine filters. Duggar and Karrer have already shown that the tobacco mosaic virus must exist in particles of extremely small size. Continuing this line of investigation, Mulvania, working in our laboratory, has repeatedly demonstrated that the tobacco mosaic virus will dialyze through certain graded collodion ultrafilters which, according to all probabilities, should not permit the passage of particles much larger than the ordinary protein molecule. One naturally wonders, therefore, if it is possible that the mosaic particles are living entities. There are those who would prefer to agree with Beijerinck that the virus is essentially a solution rather than a suspension, while still others prefer not to think of it as a virus at all in the sense that it reproduces itself, but suggest that the causal agency may be of the nature of an auto-catalytic agent. At any rate, the burden of proof as to the approximate size of the virus particles does not seem to lie with those who contend that the causal agency exists in extremely small particles. While certain protozoa and bacteria may pass through antibacterial filters bodily or fragmented, it is extremely unlikely that such bodies will dialyze through collodion membranes.

INTRA-CELLULAR BODIES

The plasmodium-like bodies, usually of surprisingly definite structure, and commonly associated with certain virus diseases in the host cells, remain puzzling. The proof of the causal relationship of these bodies to the disease has, however, always seemed insurmountable in view of the extremely small size of the infectious mosaic particles, unless a mode of reproduction heretofore unknown is assumed. To those who are still concerned with their possible causal relationship, it may be of interest to know that continued cytological observations by Miss Ismè Hoggan in our laboratory indicate that, while these bodies are readily found in a number of different hosts infected with the tobacco mosaic virus, they apparently are lacking in these same hosts infected with other virus diseases producing quite similar symptoms. These results suggest that no necessary relationship exists between the intra-cellular bodies and the occurrence of virus diseases. It is most probable that the intra-cellular bodies are a result rather than a cause of the disease. There seems to be some correlation, however, between the occurrence of these bodies and the ability of the virus to survive for long periods outside the living plant.

ARTIFICIAL CULTURES

Almost all investigators of virus diseases begin with an attempt at culturing the causal agency but are for the most part rapidly discouraged despite the number of partial successes reported. A year ago Olitsky reported on what appeared to be a good basis that the culturing of a mosaic virus had been accomplished. Mulvania tried to repeat this in our laboratories without success, and I understand others also have failed. For the present at least this phase of the problem also must remain unsettled, since negative evidence is rarely wholly convincing.

In conclusion, the results of the recent investigations on the viruses secured from apparently healthy potatoes may be mentioned briefly. The main results of this work have been published so recently that little important new data has been secured. It has been suggested by this work that either potatoes are exceptionally good examples of symptomless carriers or that normal or abnormal protoplasm of the potato itself is capable of attacking, as it were, the tobacco plant. I am indebted to Dr. H. H. Whetzel for the suggestion of the word "viroplasm" to cover the meaning of the latter working hypothesis. While this hypothesis may be weak from one point of view, it has the advantage of explaining practically all that is definitely known regarding the properties and behavior of plant viruses. However, one of these viruses of the type secured from some apparently healthy potatoes which we have referred to as "spot-necrosis" on tobacco is capable of causing a serious and even fatal disease when inoculated back to the variety of potatoes from which it came. The virus may remain dormant in the tubers for months, as in the case of other virus diseases of the potato. An explanation of this phenomenon on the basis of increase and decrease in virulence of the virus does not appear wholly to account for the facts. Something has occurred in this process of transference from one plant to another which suggests that we are working close to the boundary line of knowledge which so effectively screens from our view the actual nature of the causal agency of filterable virus diseases. If any significance at all can be attached to the viewpoints and experiments mentioned in this paper, they seem to suggest at least that the causal agency of virus diseases of plants is in some way closely related biologically to the living matter of green plants.

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PHYTOPATHOLOGICAL NOTES

Factors affecting the properties of a virus.—Experiments carried out with tobacco mosaic show that virus of low concentration has a lower thermal destruction point than a concentrated virus. Diluted virus also loses its potency faster than concentrated virus on standing at room temperature.

When tobacco and cucumber juices were used as diluting media, the potency of the virus and its thermal destruction point was depressed more than when sterile distilled water was used for making dilutions. Cucumber juice had an especially marked depressing effect on the thermal inactivation point of the virus, and this juice also seemed more effective in reducing the potency of the virus on standing.

These studies suggest that the first reduction in the potency of diluted virus is not always permanent, as, in some cases, an increase in potency was found on the sixth and twelfth days after dilution. However, on standing 50 days, there seemed to be a decrease in potency below any previous low point. Additional study is necessary on this phase in order to distinguish positively between what may be experimental variation and reactivation of previously inactivated virus through colloidal and adsorption changes, or an actual increase in the number of virus particles not previously inactivated.—H. H. McKinney, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Wisconsin Agricultural Experiment Station, Cooperating.

Authority.—The following quotation furnishes the authority for, and the definition of, a very useful word in plant pathology—a word which either has not yet found its way into the dictionary or else is mentioned at the bottom of the page. The authority is the distinguished Belgian academician, Ch. Morren, who was perhaps the first to champion the mycetogenetic origin of potato blight. The complete citation is as follows: Morren, Ch. Des causes des disettes en céréales, depuis le commencement du XIX siècle. Bul. Acad. Roy. Sci. Bruxelles **20**[3]: 169–200. 1853. The article is reprinted in Morren's *Clusia*, Liége, 1852–1874, on the second pages (29) to (60). On page 174 [second page (34) of *Clusia*] one may find the following paragraph:

"On peut raisonnablement admettre qu'en prenant l'agriculture depuis le commencement du siècle, les sciences naturelles se soient assez perfectionées pour qu'elles aient pu faire une distinction entre les disettes produites par les phénomènes météorologiques et celles dont la cause dépend d'une véritable épidémie d'animaux ou de plantes nuisibles. Si l'on pouvait créer des mots à volonté pour rendre ses idées, le nom

d'*épizootie*, exprimant une maladie quelconque attaquant le bétail dans son ensemble, devrait trouver un mot correspondant à l'égard des plantes cultivées pour les récoltes. Le nom d'*epiphytotie* serait assez convenable pour exprimer ces maladies générales des plantes utiles. Ce serait alors à l'étude des causes de ces *épiphytoties* qu'il fraudrait se livrer.''

The statement is also of particular significance when it is recalled that, with the exception of Payen in France and Berkeley in England, the scientists of the time had been saying for the past nine years that *Botrytis* was the result of the potato disease, not the cause of it.—D. REDDICK.

ABSTRACTS OF PAPERS READ AT THE SEVENTH ANNUAL MEETING OF THE CANADIAN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, ST. CATHARINES, ONTARIO, DECEMBER 21 AND 22, 1925

Studies on the epidemiology of wheat stem rust in Manitoba, 1925. D. L. BAILEY and J. H. CRAIGIE.

Slides were exposed at various intervals from the first of July to the end of September by aeroplane at elevations of 3,000 to 5,000 feet on the Royal Canadian Air Force patrols at Victoria Beach, Norway House, and the Pas in Manitoba, and at High River in Alberta. When the results of these exposures were correlated with field observations, the indications were that rust spores had been carried by the wind for considerable distances from their place of origin.

Corrosive sublimate and time of treatment in relation to yield and control of Rhizoctonia. J. E. HOWITT and W. G. EVANS.

The results of six years' experiments in treating seed potatoes with different strengths of corrosive sublimate, and for varying periods of immersion, may be summarized as follows.

1. In Ontario, the strengths of corrosive sublimate and times of immersion usually recommended, namely corrosive sublimate 1 part by weight to 2,000 of water for 3 hours, corrosive sublimate 1 part by weight to 1,000 of water for 2 hours, and 1 part by weight to 1,200 of water for 1 hour, do not reduce the amount of *Rhizoctonia* sufficiently to insure satisfactory seed potatoes.

2. Much stronger solutions of corrosive sublimate than have been usually recommended can be used with safety if the disinfection is done very early in the spring when the potatoes are completely dormant; but, if potatoes are not completely dormant at time of disinfection, strong corrosive sublimate solutions cannot be used safely, as they retard the development of the sprouts and reduce the yield. In the experiments recorded, in a 5-year test, corrosive sublimate, of the strength of 1 part by weight to 500 of water for 2 hours, practically eliminated disfigured tubers, the average percentage for the period of 5 years being only 2.2 per cent. The average percentage in the check for a period of 6 years was 68.43 per cent.

3. Entirely satisfactory results cannot be obtained by disinfecting seed potatoes with any strength of corrosive sublimate after the eyes have started to grow. Strong solutions delay sprouting of the seed pieces and reduce the yield; and weak solutions, while they apparently increase the yield of salable tubers, do not sufficiently reduce the amount of disfigurement due to sclerotia to make the potatoes desirable for seed purposes.

Verticillium "wilt" of the raspberry. G. H. BERKELEY and A. B. JACKSON.

This disease was first reported by the authors in 1923 as "Bluestem." It is now considered advisable to discard this term for "wilt." The symptoms are a wilting and casting of the leaves, with an accompanying bluish discoloration of the cane. This disease is general throughout Ontario and has been found on Cuthbert, St. Regis, Marl-

boro, and Viking varieties. The disease is caused by a previously undescribed species of *Verticillium* which the authors have named **Verticillium ovatum** Berkeley and Jackson. A description is given.

The authors also point out that the blackening of cultures of *Verticillium* spp. is caused by sclerotia in some cases, whereas in others it is produced by resting mycelium. On this basis, the forms are divided into two groups: Group A, the resting mycelial type; and Group B, the sclerotial type.

Some observations on ascospore discharge and dispersal of conidia of Venturia inequalis (Cooke) Winter. J. E. HOWITT and W. G. EVANS.

Observations and experiments carried on for a period of six years indicate the following.

Apparently the average mean temperature of the months of January, February and March has more influence upon ascospore development and primary discharge of ascospores in the spring than has the amount of precipitation from April 1 to primary ascospore discharge.

The average mean temperature for January, February and March is more closely related to ascospore development and discharge than is the mean temperature of the days in April and May preceding primary ascospore discharge.

Free ascospore discharge takes place in many seasons at the time that the leaf buds are just bursting and the blossom clusters pushing up, emphasizing the importance of the delayed dormant and pre-pink sprays in some years.

Ascospores in Ontario may retain their vitality, at least until the eighth of August, and probably until a much later date.

The conidia may be scattered freely by wind from tree to tree in an orchard.

Some fungus foes of the flower garden. A locality list with notes. J. DEARNESS.

Aster: Rust—*Coleosporium solidaginis* (Schw.) Thum.; not severe.
Yellows—organism undetermined; destructive.
Wilt—*Fusarium conglutinans callistephi* Beach; destructive.
Carnation: Rust—*Uromyces caryophyllinus* (Schrank.) Wint.
Chrysanthemum: *Cylindrosporium chrysanthemi* Ell and Dearn.
Cosmos: Wilt—undescribed.
Foxglove: Leaf-spot—*Phyllosticta digitalidis* Bellynck; injurious.
Geranium: Wilt—*Bacterium pelargonii* Brown, or *B. erodii* Lewis; destructive.
Gladiolus: Dry Rot—undetermined.
Hollyhock: Leaf-spot—*Cercospora althaeina* Sacc.; injurious.
Rust—*Puccinia malvacearum* Bert.; disfiguring and injurious.
Honeysuckle: Powdery mildew—*Microsphaera alni lonicerae* (D. C.) Salm.
"Die-back"—not determined.
Iris: Root rot—probably *Bacillus carotovorus* Jones; destructive.
Leaf-spot—*Didymellina iridis* (Desm.) Tisdale; injurious.
Paeony: Blight—*Botrytis* probably both *cinerea* Pers. and *paeoniae* Oud.
Phlox: Powdery mildew—*Erysiphe cichoracearum* D. C.
Rose: Powdery mildew—*Sphaerotheca pannosa* (Wallr.) Lev.; mostly *Oidium*.
Black leaf-spot—*Diplocarpon rosae* Wolf; common and injurious.
Rusts—*Earlea speciosa* (Schw.) Arth; *Phragmidium disciflorum* (Tode) James; *P. americanum* Diet.
Cankers—*Diaporthe umbrina* and *Coniothyrium* are to be looked for.
Snapdragon: Rust—*Puccinia antirrhini* Diet. & Holw.; transplanted from greenhouse to gardens but not sure to survive winter.

Sunflower: Rust—*Puccinia helianthi* Schw.; disfiguring and injurious.
Sweet Pea: Root rots—*Thielavia basicola* (B. and B.) Zopf.
Tulip: Blight—*Botrytis tulipae* (Lib.) Hopkins.
Violet: Root rot—*Thielavia basicola* (Berk. and Br.) Zopf.
Leaf-spot—*Cercospora violae* Sacc. on the English violet.

The dry rot disease of gladioli. F. L. DRAYTON.

Repeated isolations made from diseased gladioli sent by growers, and intercepted at the ports of entry in shipments from Holland, yielded a fungus which proved pathogenic and was later found to be identical with cultures received from Dr. Massey, of Cornell University, as the fungus causing the dry rot disease described by Mr. Errett Wallace in an unpublished thesis in 1909.

The potential importance of this disease is shown by the results of field experiments in which an average of 80 per cent, and in some varieties 100 per cent, infection was obtained, with checks remaining healthy.

The premature death and decay of diseased plants in the field and the presence of lesions and other symptoms on the diseased corms are described.

The causal fungus is quite distinct from *Septoria gladioli* Pass., the cause of the hard rot disease. Numerous small sclerotia are produced in culture and on the base of the leaf sheaths in diseased plants, but no sports have as yet been found. The mycelium is intracellular in its development and is accompanied by a disappearance of the starch and the formation of a layer of cork cambium which isolates the lesions. The vascular bundles are sometimes invaded also and become black.

For the control of this disease formalin, mercuric chloride, Uspulun, and Bayer Compound were used as corm treatments and soil drenches without success. The following recommendations are made however:

1. The strict avoidance of land which has borne a diseased crop for further gladiolus culture; also care to avoid the transference of soil from diseased to clean land.

2. Corms having lesions should not be planted. To be sure of the absence of lesions, the corm scales should be removed, if possible, just before planting.

3. Plants showing signs of disease during growth or at digging time should be discarded, for, if the cormels from diseased plants get mixed with those from healthy ones, it is impossible to separate them, and they become a source of infection when planted.

4. When cleaning the corms, it is possible to detect diseased corms and eliminate them.

5. Healthy corms selected from a mixture of diseased and healthy ones should be soaked in 5 per cent formalin for 10 minutes before they are planted.

6. Rigid inspection of corms should be made at the ports of entry.

7. Inspection and certification of corms, when the development of industry demands it, would aid materially in the control of gladiolus diseases. No decided resistance was observed, but the Primulinus hybrids used in these experiments were more susceptible than the Glandavensis types.

The work is not complete, but is reported largely to direct the attention of plant pathologists and growers to the disease and the recommendations for control based on field and laboratory studies.

A preliminary note on Fomes pinicola Fr. and Pholiota adiposa Fr.—two heterothallic species of wood-destroying fungi. IRENE MOUNCE.

No cytological investigation has hitherto been made of the mycelium of *Fomes pinicola* or *Pholiota adiposa*. The absence of clamp-connections from monosporous

mycelia and their presence in compound mycelia resulting from the pairing of monosporous mycelia are the only criteria which have been employed in determining that these two species of wood-destroying fungi are heterothallic.

It has also been found (a) that *Fomes pinicola* and *Pholiota adiposa* are bisexual; (b) that in both species (1) monosporous mycelia either remain wholly sterile or produce imperfect fruit-bodies which have never shed spores; on the other hand those compound mycelia formed by the union of two monosporous mycelia produce normal sporophores. (2) Monosporous mycelia isolated from spores of fruit-bodies from different localities are mutually fertile.

New and unusual diseases and injuries of tobacco. C. M. SLAGG.

The Fusarium leaf- and stem-disease of tobacco has been noted in the seedbeds and fields in Kentucky, Wisconsin, Connecticut, and Massachusetts since 1918, but the damage caused is slight. The curly dwarf disease, characterized by the stunting, rosetting, and leaf spotting of affected plants, has been observed in Connecticut, Ontario, and Wisconsin since 1922. In extreme cases, as high as 20 per cent of plants have been found affected. The cause is unknown. Injury from the residual effects of a blasting material known as "borrowite" occurred on tobacco plants at Harrow, Ontario, in 1925. A type of stunting and malformation of large field plants in Ontario and Quebec in 1924 was found to be caused by attack by the common burdock borer *Papaipema nitella* Gn. During the growing season of 1925 in the Okanagan Valley, British Columbia, a type of irrigation injury was observed showing sunken, longitudinal stalk cankers, abnormal defoliation, and, in some cases, frenching symptoms.

Experiments for the control of onion smut. D. R. SANDS.

In some parts of the onion growing marshes in southwestern Ontario, smut is causing considerable loss in the crop. Formalin and Kalamit were tried at various strengths, the object being to get a solution that would give the best results with the minimum quantity of water. A determination of the moisure content of the soil (black muck) was not made, but at the time of seeding and for a long period afterwards, the soil was much drier than usual. Results are given in the following table.

TABLE 1.—*The effect of solutions of formaldehyde and Kalamit on yield of onions in Ontario*

Strength of solution		Gallons per acre	Yield per acre in bu.
Solution used	Water in gallons		
1 pt. formalin	16	130	568
do	12	100	540
do	6	50	450
1 pt. Kalamit	12	100	522
do	6	50	440
Check rows—no treatment			198

In all of the above solutions, about the same quantity of formalin and of kalamit was used per acre; hence it will be seen that the best results were obtained where the greatest quantity of water was used. Rows were 16 inches apart. The measure was the imperial gallon (10 lbs. water) and imperial pint (20 oz.).

TITLES AND ABSTRACTS OF PAPERS READ AT THE TENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, MILLS COLLEGE, OAKLAND, CALIFORNIA, JUNE 16–19, 1926

Transpiration as a pathological factor.—R. E. SMITH.

Observations on citrus diseases in South Africa.—H. J. WEBER.

Plant diseases in 1925.—R. J. HASKELL.

Cytology of root tips from sugar beets having the curly-top disease.—T. E. RAWLINS.

A degeneration of the pericycle cells and several rows of adjacent cells was regularly observed in root tips from sugar beets having the curly-top disease. The first evidence of degeneration was seen in the abnormal staining reactions of nuclei in the pericycle cells. This abnormality was apparently followed by the development of a coarse reticulum in the nucleus, the disappearance of a distinct nucleolar vacuole, the occasional division of the nucleole into several parts, and the appearance of a dense reticulate cytoplasm in the degenerating cells.

Several types of heavily staining cell inclusions were quite regularly found in the cytoplasm of these degenerating cells. Some of the inclusions were irregular in form, had a dense homogeneous internal structure, and assumed no regular position in the cytoplasm. Others were more or less spherical in form, had a heterogeneous internal structure, and were usually found at each end of the cell.

The amorphous nature of all of the observed inclusions indicates that they are products of the disease rather than causal organisms.

What is "sore-shin"?—MICHAEL SHAPOVALOV.

The beginning of the cotton growing seasons of 1925 and 1926 in the Southwest was marked by a severe outbreak of brown stem lesions on the seedlings. The injury had an appearance of typical "sore-shin," but isolations from the lesions showed that a great proportion of them was not due to *Rhizoctonia*. Specimens from a southern part of the San Joaquin Valley yielded cultures of *Rhizoctonia* as well as *Fusarium*. Farther north mostly *Fusarium* spp. were obtained. Material from the Palo Verde Valley and the Yuma section gave in part *Rhizopus* sp. and in part *Fusarium* sp. At Riverside most of the lesions were due to *Fusarium*. Other miscellaneous fungi have been obtained, but in less significant numbers.

This field survey and subsequent greenhouse inoculations indicate that the cause of cottom stem lesions may vary in accordance with the soil fungous flora in different sections and with climatic changes in the same section. The common name "sore-shin" may be convenient in designating a general group of cotton stem lesions similar in external characteristics, but it may readily lead to confusion if used to signify only the injuries caused by *Rhizoctonia solani*.

Further studies on avocado fruit decays.—WM. T. HORNE.

Studies of avocado fruit rots have been continued. The outstanding importance of *Rhizopus* spp. continues to be evident. A feature of the rot not previously emphasized is that the limits of decay are not well marked, so that affected fruit can not readily be trimmed and the sound part used. Rhizopus rotted fruit has been fed to guinea pigs at the rate of five grams per animal, and ten grams have been fed to a rabbit, without injury. A rather tough elastic rot has been produced by inoculation with *Pythiacystis citrophthora* S. and S. The rot is firmer, finer textured, less moist and slower acting than Rhizopus rot. The blue and green molds of citrus fruits [*Penicillium italicum* Wehm. and *P. digitatum* (Fr.) Sacc.] have not been destructive on avocado in our inoculations, but the blue mold of apples, *P. expansum* Lk., has caused a rot which advances less rapidly and is dryer in texture than on apples. *Sclerotinia libertiana* Fcl. and *Botrytis vulgaris* Fr. produce extensive wine colored rots on dark fruit in artificial infection, but *Scl. cinerea* (Bon.) Schrot. from apricot has not grown well on avocado fruit.

Notes on some tropical anthracnoses.—WM. T. HORNE.

Studies have been carried on at Berkeley and field observations have been made in Florida and Cuba on anthracnose of lime (*Citrus aurantifolia* Sw.), mango (*Mangipera indica* L.), and anonaceous fruits, including the anon of sugar apple (*Anona squamosa* L.), and the soursop (*Anona muricata* L.). The work of Clausen[1] on *Gloeosporium limetticolum* has been confirmed in that this fungus causes anthracnose on young tissues of lime but not on other ordinary citrus species. Limes were also inoculated with the other fungi mentioned in this abstract, but anthracnose was not produced by them. A *Colletotrichum* was isolated from young mango fruits from Florida and Cuba showing typical anthracnose. This fungus produced typical anthracnose on mango foliage in the greenhouse. Other *Colletotrichums* and *Gloeosporium limetticolum* failed to give typical mango anthracnose, though flecking and staining of wound edges were produced.

A *Colletotrichum*-like fungus was isolated from mature fruits of soursop. It is distinct in culture from the fungi mentioned above. We did not succeed in producing typical anthracnose on young foliage of cherimoya (*Anona cherimolia* Mill.), nor on soursop, though some injury was caused in young soursop plants under extremely moist conditions in several of the inoculations.

Mutation in fungi.—O. A. PLUNKETT.

A study of 39 species of fungi belonging to 18 genera has shown mutation to occur in four genera and seven species, giving rise to 17 mutants. The fungi were all grown from single spores and the purity of all cultures thus guaranteed. Mutations appear as sectors in a normal colony. Mutation is evidenced in Alternaria species in general colony characters; rate of growth; conidial production; conidial length, breadth, and septation; mycelial characters, color, zonation, and presence or absence of aerial mycelium. Two mutants derived from a single-spore culture of *Sterigmatocystis niger* differ from the original essentially in the size of the spore heads and the length of the conidiophores. Five distinct mutants were derived from a single-conidial culture of *Colletotrichum lagenarium*. These mutants differ in general colony characters; production and position of acervuli; mycelial characters, color, zonation, density of growth, sclerotia, and presence or absence of aerial mycelium. Five isolations of *Glomerella cingulata* from various sources gave three cultures which mutated and two which did not.

[1] Clausen, R. E. A new fungus concerned in wither tip of varieties of *Citrus medica*. Phytopath. 2: 217–234. 1912.

From these three strains six mutants were obtained, only two of which appear identical in culture. Mutation is evidenced in these races of *Glomerella cingulata* on agar in general colony characters; production of acervuli; production and kinds of perithecia; mycelial characters, color, zonation, and presence or absence of aerial mycelium. On apples the mutants differ in color of spot produced; general surface appearance of spot; nature of rot; rate of rotting; abundance and position of acervuli; abundance and kind of perithecia; abundance of aerial or surface mycelium.

All of the mutants are permanent in character and do not revert to the original form even when grown for a long time on their normal host. Certain apparent reversions occur in sectors of mutant colonies, but the whole colony never changes. Mutants may give rise to further mutations. Experimental attempts to produce mutation artificially and to secure new races of fungi by selection have failed. Modifications produced by environmental changes, poisons, modified media, etc., revert to the original form when grown again under normal conditions. Mutation is common in certain fungi in culture and possibly is responsible for new races and strains in nature.

Single-spore isolation simplified.—H. N. HANSEN.

Glass capillaries slightly greater in diameter than the spores to be isolated are filled by capillarity from a spore suspension made in warm nutrient agar. After a few trials a spore dilution giving from 1 to 4 spores to a tube 3 cm. long is easily made. The filled capillaries are then examined under the microscope and broken up according to the number and position of the spores observed. The resultant pieces are again examined to make sure that each contains one spore only, then picked up with forceps, immersed in alcohol to sterilize the outside and placed in the desired medium. By using solid rather than liquid medium the capillaries may be broken without disturbing the contents and sterilized without injury to the spores. If a culture from a single hyphal tip is desired, the piece of glass tube with the spore is pressed to the bottom of the medium in a petri dish, where germination may be followed under the microscope. When the germinating hypha has grown beyond the end of the tube, it is cut off and the glass removed. A single hyphal tip is easily obtained in this manner because of the microscopic size of the tube.

Transpiration of healthy and blighted tomato plants (Western yellow tomato blight).—MICHAEL SHAPOVALOV.

It has been shown previously by the writer that a close and direct correlation exists between the rate of evaporation and the progress of Western tomato blight. The environment, whether natural or artificial, which tends to reduce the rate of evaporation from atmometers and the rate of transpiration from the plants is likely to reduce also the percentage of blight. It may have been expected that varieties possessing a greater transpiratory power would be more susceptible to blight than those which transpire less, but this does not necessarily appear to be the case. Dwarf Champion, which is generally recognized as more resistant to Western blight than Stone, shows a higher rate of transpiration than does the latter. A few other varieties behave similarly.

The rate of transpiration of blighted plants progressively decreases as the disease advances toward its last stage, culminating in a pronounced yellowing and death of the plant.

The transpiration data have been obtained with growing plants by means of the cobalt-chloride paper method as well as with cut shoots in potometers.

Breeding lettuce for tip-burn resistance.—J. B. SCHERRER and T. E. RAWLINS.

Breeding and selection work was begun in an endeavor to develop a tip-burn resistant head lettuce.

Iceberg variety, while not immune, shows considerably more resistance to tip-burn than the New York variety. Iceberg and New York were crossed in an attempt to develop a variety having the desirable characteristics of New York and the tip-burn resistance of Iceberg. The red color of Iceberg was found to be dominant, all of the F_1 plants and approximately three fourths of the F_2 plants showing some red color. The F_2 plantings contained about one-half as many tip-burned plants as the New York planting, and most of the tip-burned F_2 plants were less severely injured than the diseased New York plants. Approximately 15 per cent of the F_2 plants were very similar to the New York variety in that they had firm heads, no red color, and the New York type of leaf texture.

Inheritance of resistance to Helminthosporium californicum in a cross between Chevalier barley, a resistant variety, and Abyssinian, a susceptible variety.—W. W. MACKIE.

Within the past few years a serious disease of barley, caused by a new fungus identified as *Helminthosporium californicum*, has appeared in California. The name rusty blotch has been given to it because of the large, rusty colored lesions appearing on the leaf blades. Among the hundreds of barley varieties examined, one, the Chevalier, a two-rowed variety, appeared immune. Crosses and reciprocal crosses between Abyssinian, a higher yielding, lodging resistant, but rusty blotch susceptible variety, and Chevalier gave plants free from rusty blotch in the F_1 generation, indicating the dominance of resistance. In the F_2 generation approximately one-fourth of the plants were attacked, with the remainder free from rusty blotch, indicating an inheritance of resistance to rusty blotch in a simple 3:1 ratio due to a single factor for resistance.

This species cross, Chevalier (*Hordeum distichum*) × Abyssinian (*Hordeum deficiens*), gave plants which segregated in a 1:2:1 ratio or one-fourth homozygous *H. distichum* plants, one-half intermediate or heterozygous plants, and one-fourth homozygous *H. deficiens* plants.

All three of these groups of segregates were found to include rusty blotch infected plants in approximately a 3:1 ratio, as was found for the F_2 group as a whole.

These ratios will be checked by plant-to-row tests in the F_3 generation.